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(54) Title: INTERLEUKIN-21 (IL-21) AND IL-21 RECEPTOR (IL-21R) MODULATION OF REGULATORY T CELLS AND  
FORKHEAD BOX P3 (FOXP3)

Fig. 1

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Human IL-21R 1 MPRGWAAPLLLLLLQCCWGCPLVCYTDYLTQVICILEMWNHNPSTLT 50
Murine IL-21R 1 MPRGPVAAALLLLLLHGAWSCLDLCYTDYLTQVICILEMWNHNPSTLT 50

Human IL-21R 51 WQDQYEELEKDEATSCSLHRSANHNATHATYTCHEMDVHFMADDIFSVNITD 100
Murine IL-21R 51 WQDQYEELEKDEATSCSLHRSANHNATHATYTCHEMDVHFMADDIFSVNITD 100

Human IL-21R 101 QSGNYSQECGSEFLAESIKPAPPFNVTFTSGQYNISNRSYEDYDFAYML 150
Murine IL-21R 101 QSGNYSQECGSEFLAESIKPAPPFNVTFTSGQYNISNRSYEDYDFAYML 150

Human IL-21R 151 RGKLQYELQYRNRGDFWAVSPRRKLISVDSRSVSLPLEFRKDSYELQV 200
Murine IL-21R 151 RGKLQYELQYRNRGDFWAVSPRRKLISVDSRSVSLPLEFRKDSYELQV 200

Human IL-21R 201 RAGPMGSSSYQCTWSEWSDPVIFQTQSEELKEGWNPHLLLLLVIFVIF 250
Murine IL-21R 201 RAGPMGSSSYQCTWSEWSDPVIFQTQSEELKEGWNPHLLLLLVIFVIF 250

Human IL-21R 251 AFWSLKHPLWRLWKKINA.VSPERFFEMPLYKCGSGDFKKNVGAFTGS 299
Murine IL-21R 251 AFWSLKHPLWRLWKKINA.VSPERFFEMPLYKCGSGDFKKNVGAFTGS 299

Human IL-21R 300 SLELGPWSPEVSTLEVYCHPPSPAKRLQLTELQEPALIVESDGVKPK 349
Murine IL-21R 300 SLELGPWSPEVSTLEVYCHPPSPAKRLQLTELQEPALIVESDGVKPK 349

Human IL-21R 350 SFW...PTAQNSGGSAYSEERDRPYGLVSDTDTVTLDAEGPCTWPCSCD 396
Murine IL-21R 350 SFW...PTAQNSGGSAYSEERDRPYGLVSDTDTVTLDAEGPCTWPCSCD 396

Human IL-21R 397 DGYPALDLDALEPSGLDPLDAGTTVLSCGCVSAGSPGLGGLSLL 446
Murine IL-21R 397 DGYPALDLDALEPSGLDPLDAGTTVLSCGCVSAGSPGLGGLSLL 446

Human IL-21R 447 DRLLKPLADGEDWAGGLPWGGRSPGVSEAGSPGLAGLMDTDFDSGFVG 496
Murine IL-21R 447 DRLLKPLADGEDWAGGLPWGGRSPGVSEAGSPGLAGLMDTDFDSGFVG 496

Human IL-21R 497 SDCSSPVECDFTSPGDEGPPRSYLRQWVV.IPPFLSSPGDQAS* 539
Murine IL-21R 497 SDCSSPVECDFTSPGDEGPPRSYLRQWVV.IPPFLSSPGDQAS* 539

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(57) Abstract: Methods of modulating the level and activity of Tregs and Foxp3 using modulators of IL-21/IL-21 R, i.e., IL-21/IL-21 R agonists (i.e., IL-21 agonists and IL-21R agonists) and IL-21/IL-21 R antagonists (i.e., IL-21 antagonists and IL-21 R antagonists), are disclosed. IL-21 /IL-21 R agonists and antagonists can be used to enhance immunity or induce immune suppression in vivo, ex vivo and/or in vitro, e.g., for treating, ameliorating or preventing autoimmune or inflammatory disorders, cancers, and infectious disorders.



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## TITLE

### **INTERLEUKIN-21 (IL-21) AND IL-21 RECEPTOR (IL-21R) MODULATION OF REGULATORY T CELLS AND FORKHEAD BOX P3 (FOXP3)**

#### Related Applications

**[0001]** This application claims the benefit of priority from U.S. Provisional Patent Application No. 61/025,586, filed February 1, 2008, the content of which is hereby incorporated by reference herein in its entirety.

## BACKGROUND OF THE INVENTION

#### Field of the Invention

**[0002]** The present invention relates to methods and compositions for modulating the levels and/or activity of regulatory T cells (Treg) and the forkhead box P3 (Foxp3) transcription factor, comprising interleukin-21 (IL-21) and/or interleukin-21 (IL-21R) agonists and antagonists. The methods and compositions disclosed herein are useful in, e.g., immunotherapy.

#### Related Background Art

**[0003]** Human IL-21 is a cytokine that shows sequence homology to IL-2, IL-4 and IL-15 (Parrish-Novak et al. (2000) *Nature* 408:57-63). Despite low sequence homology among interleukin cytokines, cytokines share a common fold into a “four-helix-bundle” structure that is representative of the family. Most cytokines bind either class I or class II cytokine receptors. Class II cytokine receptors include the

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receptors for IL-10 and the interferons, whereas class I cytokine receptors include the receptors for IL-2 through IL-7, IL-9, IL-11, IL-12, IL-13, and IL-15, as well as hematopoietic growth factors, leptin, and growth hormone (Cosman (1993) *Cytokine* 5:95-106).

**[0004]** Human IL-21 receptor (IL-21R) is a class I cytokine receptor that is expressed in lymphoid tissues, in particular by NK, B and T cells (Parrish-Novak et al. (2000) *supra*). The nucleotide and amino acid sequences encoding human interleukin-21 (IL-21) and its receptor (IL-21R) are described in, e.g., WO 00/53761; WO 01/85792; Parrish-Novak et al. (2000) *supra*; and Ozaki et al. (2000) *Proc. Natl. Acad. Sci. U.S.A.* 97:11439-44. IL-21R has the highest sequence homology to the IL-2 receptor  $\beta$  chain and the IL-4 receptor  $\alpha$  chain (Ozaki et al. (2000) *supra*). Upon ligand binding, IL-21R associates with the common gamma cytokine receptor chain ( $\gamma_c$ ) that is shared by receptors for IL-2, IL-3, IL-4, IL-7, IL-9, IL-13 and IL-15 (Ozaki et al. (2000) *supra*; Asao et al. (2001) *J. Immunol.* 167:1-5). The widespread lymphoid distribution of IL-21R suggests that IL-21 plays an important role in immune regulation. Indeed, *in vitro* studies have shown that IL-21 significantly modulates the function of B cells, CD4<sup>+</sup> and CD8<sup>+</sup> T cells, and NK cells (e.g., Parrish-Novak et al. (2000) *supra*; Kasaian et al. (2002) *Immunity.* 16:559-69).

**[0005]** IL-21R is expressed in lymphoid tissues, particularly on T, B, NK, dendritic cells (DC) and macrophages (Parrish-Novak et al. (2000) *supra*), which allows these cells to respond to IL-21 (Leonard and Spolski (2005) *Nat. Rev. Immunol.* 5:688-98). Recent evidence suggests that IL-21-mediated signaling can have antitumor activity (Sivakumar et al. (2004) *Immunology* 112:177-182), and that IL-21 can prevent antigen-induced asthma in mice (Shang et al. (2006) *Cell. Immunol.* 241:66-74).

**[0006]** IL-21 can promote Th2 cell function (Pesce et al. (2006) *J. Clin. Invest.* 116:2044-55; Strengell et al. (2002) *J. Immunol.* 169:3600-05; Wurster et al. (2002) *J. Exp. Med.* 196:969-77), can cooperate with IL-15 in enhancing the expansion and activity of CD8<sup>+</sup> T cells (Zeng et al. (2005) *J. Exp. Med.* 201:139-48) and NK cells (Strengell et al. (2003) *J. Immunol.* 170:5464-69; Zeng et al. (2005) *supra*), and can

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enhance the survival and proliferation of NKT cells (Coquet et al. (2007) *J. Immunol.* 178:2827-34). IL-21 can induce B cell apoptosis or, along with IL-4, stimulate B cell proliferation to promote differentiation toward memory and/or plasma cells (Ettinger et al. (2005) *J. Immunol.* 175:7867-79; Mehta et al. (2003) *J. Immunol.* 170:4111-18).

[0007] In autoimmunity, disruption of the IL-21 gene or injection of recombinant IL-21 have been shown to modulate the expression of experimental autoimmune myasthenia gravis (EAMG) and experimental autoimmune encephalomyelitis (EAE), respectively (King et al. (2004) *Cell* 117:265-77; Ozaki et al. (2004) *J. Immunol.* 173:5361-71; Vollmer et al. (2005) *J. Immunol.* 174:2696-701; Liu et al. (2006) *J. Immunol.* 176:5247-54). In these experimental systems, it has been suggested that the manipulation of IL-21-mediated signaling directly altered the function of CD8<sup>+</sup> cells, B cells, T helper cells, and NK cells. Some of the roles of IL-21/IL-21R in immune modulation have previously been elucidated in U.S. Patent Nos. 6,057,128; 7,189,400; 7,198,789; and 7,314,623; and U.S. Patent Publication Nos. 2004/0016010; 2004/0265960; 2006/0024268; 2006/0039902; 2006/0159655; and 2006/0257403, each of which is incorporated by reference in its entirety herein.

[0008] It is widely accepted that the pathogenic effects of autoreactive T effector cells in EAE are highly influenced by regulatory cells. One such regulatory cell population is CD4<sup>+</sup>CD25<sup>+</sup> cells, which express the forkhead transcription factor Foxp3 and contribute to the maintenance of peripheral immune tolerance (Sakaguchi (2004) *Annu. Rev. Immunol.* 22:531-62; Ziegler (2006) *Annu. Rev. Immunol.* 24:209-26). The maturation of these regulatory cells is highly dependent on IL-2 (Thornton et al. (2004) *J. Immunol.* 172:6519-23). IL-21R has some structural homology with IL-2R, as it shares a common  $\gamma$ -chain (Leonard and Spolski (2005) *supra*; Ozaki et al. (2000) *supra*).

[0009] Recently, IL-21 has been shown to counteract the effects of CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells ("Treg cells" or "Tregs") (Peluso et al. (2007) *J. Immunol.* 178:732-39). Additionally, dendritic cells (DCs) expanded in the presence of IL-21

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have an immature phenotype and inhibit T cell responses, in contrast to IL-15-generated DCs that preferentially stimulate T cell responses (Brandt et al. (2003) *J. Invest. Dermatol.* 121:1379-82); Leonard and Spolski (2005) *supra*).

[0010] The broad effects of IL-21 on diverse lymphocyte populations suggest that IL-21 and IL-21R likely play a prominent role in inflammation and immune responses.

#### SUMMARY OF THE INVENTION

[0011] Disclosed herein are findings based upon, *inter alia*, the blockade of IL-21 *in vivo* by using soluble IL-21R Fc in experimental autoimmune encephalomyelitis (EAE) -induced disease in SJL/J mice (Pesce et al. (2006) *supra*). Blockade of IL-21 before and after the induction of EAE enhances the influx of inflammatory cells into the central nervous system (CNS), as well as the severity of EAE. Furthermore, the blockade of IL-21 reduces the number of regulatory T cells ("Treg cells" or "Tregs") and downregulates the expression of the forkhead box P3 (Foxp3) transcription factor. Similar effects are seen in IL-21R<sup>-/-</sup> mice with EAE. Tregs from mice receiving IL-21R Fc also lose the ability to prevent EAE induced with PLP<sub>139-151</sub>-reactive T cells.

[0012] Additionally, IL-21R deficiency is associated with a temporary expansion of Th1 and Th2 responses (particularly Th1), as shown by increased expression of IFN- $\gamma$ , IL-4, and IL-10 in CD4<sup>+</sup> and CD8<sup>+</sup> T cells of IL-21R<sup>-/-</sup> mice. IL-21R<sup>-/-</sup> mice also showed reduced numbers of splenic NK cells in the early stage of EAE, and increased numbers of CNS NK cells during later stages of the disease. These data provide evidence that the IL-21/IL-21R pathway modulates Treg and NK cells and Foxp3 expression.

[0013] Included herein are methods and compositions for modulating the level, activity, and/or signal transduction of an IL-21 and/or an IL-21R, and/or an interaction between an IL-21 and an IL-21R, e.g., using an agonist(s) (also referred to herein as "IL-21/IL-21R agonists"), which enhance the level, activity, and/or

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signal transduction of an IL-21 and/or an IL-21R, and/or an interaction between an IL-21 and an IL-21R, or an antagonist(s) of IL-21 and/or IL-21R (also referred to herein as "IL-21/IL-21R antagonists"), which decrease the level, activity, and/or signal transduction of an IL-21 and/or an IL-21R, and/or an interaction between an IL-21 and an IL-21R. As used herein, "IL-21/IL-21R antagonists" refers to both IL-21 antagonists and IL-21R antagonists, while "IL-21/IL-21R agonists" refers to both IL-21 agonists and IL-21R agonists.

**[0014]** One aspect of the present invention provides a method of treating, ameliorating, or preventing an autoimmune disorder, an inflammatory disorder, transplant/graft rejection, lymphopenia, or graft-versus-host disease (GvHD) in a mammalian subject, comprising administering to the subject an IL-21/IL-21R agonist selected from the group consisting of agonistic IL-21/IL-21R polynucleotides or fragments thereof, agonistic IL-21/IL-21R polypeptides or fragments thereof, agonistic anti-IL-21/IL-21R antibodies or fragments thereof, and agonistic small molecules, in an amount sufficient to increase the level and/or activity of a Treg cell or a population of Treg cells in the mammalian subject, thereby treating, ameliorating, or preventing the autoimmune disorder, inflammatory disorder, transplant/graft rejection, lymphopenia, or GvHD in the mammalian subject. Preferably, the mammalian subject is a human.

**[0015]** Preferably, the agonistic anti-IL-21R antibody or fragment thereof is capable of binding to an IL-21R comprised of an amino acid sequence at least 90% identical to the amino sequence set forth in SEQ ID NO:2, wherein the IL-21R is capable of binding an IL-21.

**[0016]** Preferably, the agonistic IL-21 polypeptide or fragment thereof comprises the amino acid sequence set forth in SEQ ID NO:9, or an amino acid sequence with at least 90% identity to the amino acid sequence set forth in SEQ ID NO:9, and is capable of binding an IL-21R.

**[0017]** Preferably, the agonistic IL-21R polypeptide or fragment thereof comprises the amino acid sequence set forth in SEQ ID NO:2, or an amino acid sequence with

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at least 90% identity to the amino acid sequence set forth in SEQ ID NO:2, and is capable of binding an IL-21.

**[0018]** As part of a nonlimiting list, the autoimmune disorder can be multiple sclerosis (MS), juvenile idiopathic arthritis, psoriatic arthritis, hepatitis C virus-associated mixed cryoglobulinemia, polymyositis, dermatomyositis, polyglandular syndrome type II, autoimmune liver disease, Kawasaki disease, myasthenia gravis, immunodysregulation polyendocrinopathy enteropathy X-linked syndrome (IPEX (syndrome)), type I diabetes, psoriasis, hypothyroidism, hemolytic anemia, thrombocytopenia, spondyloarthritis, Sjögren's syndrome, rheumatoid arthritis, inflammatory bowel disease (IBD), Crohn's disease, ulcerative colitis, eczema, gastritis, or thyroiditis. As part of a nonlimiting list, the inflammatory disorder can be contact hypersensitivity or atopic dermatitis.

**[0019]** Another aspect of the present invention provides a method of transplanting/grafting an organ, tissue, cell, or group of cells to a mammalian subject comprising the steps of: (a) administering to the subject an IL-21/IL-21R agonist selected from the group consisting of agonistic IL-21/IL-21R polynucleotides or fragments thereof, agonistic IL-21/IL-21R polypeptides or fragments thereof, agonistic anti-IL-21/IL-21R antibodies or fragments thereof, and agonistic small molecules, in an amount sufficient to reduce the risk of transplant/graft rejection, and (b) transplanting/grafting an organ, tissue, cell or group of cells to the subject, either before, during, or after the administering step (a).

**[0020]** The organ, tissue, cell, or group of cells transplanted/grafted includes, but is not limited to, heart, kidney, liver, lung, pancreas, bone marrow, cartilage, cornea, neuronal tissue, and/or cells thereof.

**[0021]** Yet another aspect of the present invention provides a method of treating, preventing, or ameliorating transplant/graft rejection in a mammalian transplant/graft recipient comprising: (a) detecting a symptom of transplant/graft rejection in a transplant/graft recipient, and (b) administering to the transplant/graft recipient an IL-21/IL-21R agonist selected from the group consisting of agonistic IL-21/IL-21R



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polynucleotides or fragments thereof, agonistic IL-21/IL-21R polypeptides or fragments thereof, agonistic anti-IL-21/IL-21R antibodies or fragments thereof, and agonistic small molecules.

[0022] The symptom(s) of transplant/graft rejection includes, but is not limited to, inflammation, decreased organ function, signs of rejection in biopsy, and/or fibrosis.

[0023] A further aspect of the present invention provides a method of treating, preventing, or ameliorating a cancer or an infectious disorder in a mammalian subject, comprising administering to the subject an IL-21/IL-21R antagonist selected from the group consisting of antagonistic IL-21/IL-21R polynucleotides or fragments thereof, antagonistic IL-21/IL-21R polypeptides or fragments thereof, antagonistic anti-IL-21/IL-21R antibodies or fragments thereof, and antagonistic small molecules, in an amount sufficient to decrease the level and/or activity of a Treg cell or a population of Treg cells in the mammalian subject, thereby treating, ameliorating, or preventing the cancer or infectious disorder in the mammalian subject. Preferably, the mammalian subject is a human.

[0024] Preferably, the antagonistic anti-IL-21R antibody or fragment thereof is capable of binding to an IL-21R comprised of an amino acid sequence at least 90% identical to the amino acid sequence set forth in SEQ ID NO:2 wherein the IL-21R is capable of binding an IL-21.

[0025] As part of a nonlimiting list, the cancer can be a solid tumor, a soft tissue tumor or a metastatic lesion, and may be breast cancer, ovarian cancer, lung cancer, leukemia, lymphoma, melanoma, colorectal cancer, or renal cancer. As part of a nonlimiting list, the infectious disorder can be caused by a bacterial, viral or parasitic infection, including, but not limited to, HIV infection, *S. mansoni* infection, hepatitis infection, Epstein-Barr virus infection, *Borrelia* infection, JC virus infection, cytomegalovirus infection, Coxsackie virus infection, papilloma virus infection, or herpes virus infection.

[0026] Preferably, the antagonistic IL-21R polypeptide is comprised of an IL-21R extracellular domain and an Fc immunoglobulin fragment. More preferable is an

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IL-21R polypeptide comprised of an amino acid sequence at least 90% identical to the amino acid sequence set forth in SEQ ID NO:14. The extracellular domain preferably comprises about amino acids 1-235, 20-235, 1-236, or 20-236 of SEQ ID NO:2.

[0027] Yet another aspect of the present invention provides a method of decreasing the transcription of HIV in a mammalian subject, comprising administering to the subject an IL-21/IL-21R antagonist selected from the group consisting of antagonistic IL-21/IL-21R polynucleotides or fragments thereof, antagonistic IL-21/IL-21R polypeptides or fragments thereof, antagonistic anti-IL-21/IL-21R antibodies or fragments thereof, and antagonistic small molecules, in an amount sufficient to decrease the level and/or activity of Foxp3 in the mammalian subject, thereby decreasing the transcription of HIV in the mammalian subject. Preferably, the mammalian subject is a human.

[0028] Preferably, the antagonistic anti-IL-21R antibody or fragment thereof is capable of binding to an IL-21R comprised of an amino acid sequence at least 90% identical to the amino acid sequence set forth in SEQ ID NO:2, wherein the IL-21R is capable of binding an IL-21.

[0029] Preferably, the antagonistic IL-21R polypeptide is comprised of an IL-21R extracellular domain and an Fc immunoglobulin fragment. More preferable is an IL-21R polypeptide comprised of an amino acid sequence at least 90% identical to the amino acid sequence set forth in SEQ ID NO:14. The extracellular domain preferably comprises about amino acids 1-235, 20-235, 1-236, or 20-236 of SEQ ID NO:2.

[0030] Another aspect of the present invention provides a method of modulating the level and/or activity of a Treg cell or a population of Treg cells in a mammalian subject, comprising administering to the subject an IL-21/IL-21R agonist or an IL-21/IL-21R antagonist in an amount sufficient to modulate the level and/or activity of the Treg cell or population of Treg cells in the mammalian subject. Preferably, the mammalian subject is a human.

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**[0031]** Preferably, the IL-21/IL-21R agonist is an agonistic IL-21/IL-21R polynucleotide or fragments thereof, agonistic IL-21/IL-21R polypeptide or fragment thereof, agonistic anti-IL-21/IL-21R antibody or fragment thereof, or agonistic small molecule.

**[0032]** Preferably, the agonistic anti-IL-21R antibody or fragment thereof is capable of binding to an IL-21R comprised of an amino acid sequence at least 90% identical to the sequence set forth in SEQ ID NO:2, wherein the IL-21R is capable of binding an IL-21.

**[0033]** Preferably, the agonistic IL-21 polypeptide or fragment thereof comprises the amino acid sequence set forth in SEQ ID NO:9, or an amino acid sequence with at least 90% identity to the amino acid sequence set forth in SEQ ID NO:9, and is capable of binding an IL-21R.

**[0034]** Preferably, the agonistic IL-21R polypeptide or fragment thereof comprises the amino acid sequence set forth in SEQ ID NO:2, or an amino acid sequence with at least 90% identity to the amino acid sequence set forth in SEQ ID NO:2, and is capable of binding an IL-21.

**[0035]** Preferably, the IL-21/IL-21R antagonist is an antagonistic IL-21/IL-21R polynucleotide or fragment thereof, antagonistic IL-21/IL-21R polypeptide or fragment thereof, antagonistic anti-IL-21/IL-21R antibody or fragments thereof, or antagonistic small molecule.

**[0036]** Preferably, the antagonistic anti-IL-21R antibody or fragment thereof is capable of binding to an IL-21R comprised of an amino acid sequence at least 90% identical to the sequence set forth in SEQ ID NO:2, wherein the IL-21R is capable of binding an IL-21.

**[0037]** Preferably, the antagonistic IL-21R polypeptide is comprised of an IL-21R extracellular domain and an Fc immunoglobulin fragment. More preferable is an antagonistic IL-21R polypeptide comprised of an amino acid sequence at least 90% identical to the sequence set forth in SEQ ID NO:14. The extracellular domain

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preferably comprises about amino acids 1-235, 20-235, 1-236, or 20-236 of SEQ ID NO:2.

[0038] Yet another aspect of the present invention provides a method of modulating the level and/or activity of Foxp3 in a mammalian subject, comprising administering to the subject an IL-21/IL-21R agonist or an IL-21/IL-21R antagonist in an amount sufficient to modulate the level and/or activity of Foxp3 in the mammalian subject. Preferably, the mammalian subject is a human.

[0039] Another aspect of the present invention provides a method for increasing the ability of a vaccine composition containing an antigen to elicit a protective immune response against the antigen in a mammalian subject, comprising administering to the mammalian subject, either simultaneously with or sequentially to the vaccine composition, an effective adjuvanting amount of an IL-21/IL-21R antagonist, such that the ability of the vaccine composition to elicit the protective immune is increased. Preferably, the mammalian subject is a human.

[0040] The antigen can be derived from a pathogen selected from the group consisting of a virus, bacterium, and protozoan. The antigen can also be derived from a cancer or a tumor, and may be expressed on the surface of a cancer cell or a tumor cell.

[0041] Yet another aspect of the present invention provides a pharmaceutical composition useful as a vaccine, comprising an antigen from a pathogenic microorganism selected from the group consisting of a viral, bacterial and parasitic microorganism, and an effective adjuvanting amount of an IL-21/IL-21R antagonist, in a pharmaceutically acceptable carrier.

[0042] A further aspect of the present invention provides a pharmaceutical composition comprising a cancer cell or tumor cell antigen in combination with an IL-21/IL-21R antagonist, in a pharmaceutically acceptable carrier.

[0043] The subject can also be administered at least one additional therapeutic agent selected from the group consisting of cytokine inhibitors, growth factor inhibitors,

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immunosuppressants, anti-inflammatory agents, metabolic inhibitors, enzyme inhibitors, cytotoxic agents, and cytostatic agents.

[0044] Additional therapeutic agent(s) may be selected from the group consisting of TNF antagonists, anti-TNF agents, IL-12 antagonists, IL-15 antagonists, IL-17 antagonists, IL-18 antagonists, IL-22 antagonists, T cell-depleting agents, B cell-depleting agents, cyclosporin, FK506, CCI-779, etanercept, infliximab, rituximab, adalimumab, prednisolone, azathioprine, gold, sulphasalazine, hydroxychloroquine, minocycline, anakinra, abatacept, methotrexate, leflunomide, rapamycin, rapamycin analogs, Cox-2 inhibitors, cPLA2 inhibitors, NSAIDs, p38 inhibitors, antagonists of B7.1, B7.2, ICOSL, ICOS and/or CD28, and agonists of CTLA4.

#### BRIEF DESCRIPTION OF THE DRAWINGS

[0045] **FIG. 1** depicts a GAP comparison of the human IL-21R protein (corresponding to the amino acid sequence set forth in SEQ ID NO:2) and the murine IL-21R protein (corresponding to the amino acid sequence set forth in SEQ ID NO:4).

[0046] **FIG. 2** depicts a multiple-sequence alignment of the amino acids of human IL-21R (corresponding to SEQ ID NO:2), murine IL-21R (corresponding to SEQ ID NO:4), and human IL-2 beta chain (corresponding to SEQ ID NO:5)(GENBANK® Accession No. AAA59143). Conserved cytokine receptor module motifs (PXPP and WSXWS (SEQ ID NO:15) motifs) are indicated by boldface type. Potential signaling regions are indicated by underlining and boldface type.

[0047] **FIG. 3** depicts a multiple-sequence alignment of the amino acids of human IL-21 ("Translation of hIL-21")(corresponding to SEQ ID NO:9), murine IL-21 ("mIL-21")(corresponding to SEQ ID NO:11), and rat IL-21 ("Translation of rat IL-21 CDS")(corresponding to SEQ ID NO:13). Residues conserved across all three species of human, mouse and rat are indicated, as is conservation in two out of three species. Overall, analysis of the alignment demonstrates a 59% identity between

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amino acids 8-153 of SEQ ID NO:9 and amino acids 1-150 of SEQ ID NO:11 and amino acids 1-150 of SEQ ID NO:13.

[0048] **FIG. 4** depicts IL-21 expression *in vivo* in EAE and control mice.

[0049] **FIGs. 5A and 5B** depict blockade of IL-21 *in vivo* enhancing EAE in SJL/J mice, as measured by clinical score. **FIG. 5C** depicts photomicrographs showing blockade of IL-21 leading to severe infiltration of inflammatory mononuclear cells in the CNS.

[0050] **FIG. 6** depicts BrdU incorporation *in vivo* after IL-21R Fc treatment of EAE mice.

[0051] **FIG. 7** depicts blockade of IL-21 restoring the Th1 and Th17 responses in EAE mice. **FIG. 7A** depicts dot plots generated after gating on lymphocytes. **FIG. 7B** depicts TNF- $\alpha$ , IL-10, and IL-17 cytokine expression in the CNS by real time (RT)-PCR.

[0052] **FIG. 8** depicts IL-21 blockade modifying the number of CD4<sup>+</sup>CD25<sup>+</sup> T cells and decreasing expression of Foxp3 in CD4<sup>+</sup>CD25<sup>+</sup> T cells. **FIG. 8A** depicts representative plots from individual mice showing the percentage of CD4<sup>+</sup>CD25<sup>+</sup> T cells gated on lymphocytes. **FIG. 8B** depicts the average percentage of CD4<sup>+</sup>CD25<sup>+</sup> cells in control (IgG2a Ab) and IL-21R Fc-treated mice. **FIG. 8C** depicts the expression of Foxp3 in relation to CD25<sup>+</sup> cells gated on CD4<sup>+</sup> cells. **FIG. 8D** depicts the mean percentage of Foxp3 and CD25 double-positive cells gated on CD4<sup>+</sup> cells in control (IgG2a Ab) and IL-21R Fc-treated mice. **FIG. 8E** depicts blockade of IL-21 reducing the frequency and expression of Foxp3 in Treg cells of naive Foxp3<sup>GFP/GFP</sup> mice.

[0053] **FIG. 9A** depicts blockade of IL-21 altering the encephalitogenic potential of T cells. **FIG. 9B** depicts cotransfer of CD4<sup>+</sup>CD25<sup>+</sup> cells from IL-21R-blockaded mice failing to protect recipients from EAE.

[0054] **FIG. 10** depicts the influence of IL-21R deficiency on the development of EAE. **FIG. 10A** depicts the clinical course of active EAE in B6 and IL-21R<sup>-/-</sup>

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mice. **FIG. 10B** depicts mononuclear cell infiltration and demyelination in the CNS of B6 and IL-21R<sup>-/-</sup> mice with EAE.

[0055] **FIG. 11** depicts the effects of IL-21R deficiency on T cell proliferation in EAE.

[0056] **FIG. 12** depicts levels of intracellular cytokines (IFN- $\gamma$  (**FIGs. 12A, 12B, 12D**), IL-4 (**FIG. 12C**), IL-10 (**FIG. 12C**), and IL-17 (**FIG. 12D**)) in B6 and IL-21R<sup>-/-</sup> mice during EAE.

[0057] **FIG. 13** depicts IL-17 production in IL-21R<sup>-/-</sup> and B6 mice with EAE.

[0058] **FIG. 14** depicts peripheral Treg cells of B6 and IL-21R<sup>-/-</sup> mice with EAE.

**FIGs. 14A and 14B** depict CD4<sup>+</sup>CD25<sup>+</sup> Treg cells. **FIGs. 14C and 14D** depict CD25<sup>+</sup>Foxp3<sup>+</sup> cells on a CD4<sup>+</sup> gated subpopulation.

[0059] **FIG. 15** depicts a comparison of suppressor activity of Treg cells from B6 and IL-21R<sup>-/-</sup> mice with EAE.

[0060] **FIG. 16** depicts the response to IL-2 of Treg cells from B6 and IL-21R<sup>-/-</sup> mice with EAE. **FIG. 16A** depicts the kinetics of IL-21 production in B6 and IL-21R<sup>-/-</sup> mice with EAE. **FIG. 16B** depicts the kinetics of IL-2 production in B6 and IL-21R<sup>-/-</sup> mice with EAE. **FIG. 16C** depicts CD4<sup>+</sup>CD25<sup>+</sup> cells of IL-2 cultures from B6 and IL-21R<sup>-/-</sup> mice with EAE. **FIG. 16D** depicts Foxp3 expression of IL-2 cultures by CD4<sup>+</sup> cells (upper panel) in relation to CD25<sup>+</sup> cells gated on CD4<sup>+</sup> cells (lower panel) from B6 and IL-21R<sup>-/-</sup> mice with EAE.

[0061] **FIG. 17** shows that IL-21R<sup>-/-</sup> NK cells do not expand early in EAE, but have suppressive activity on disease. **FIGs. 17A and 17B** depict NK cells from spleens of B6 and IL-21R<sup>-/-</sup> mice with EAE. **FIG. 17C** depicts NK cells in CNS-infiltrating cells from B6 and IL-21R<sup>-/-</sup> mice with EAE. **FIG. 17D** depicts the clinical course of EAE in control-treated and anti-NK1.1 mAb-treated IL-21R<sup>-/-</sup> mice.

#### DETAILED DESCRIPTION OF THE INVENTION

[0062] Methods and compositions for modulating the level, activity, and/or signal transduction of an IL-21 and/or an IL-21R, and/or an interaction between an IL-21

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and an IL-21R, e.g., using agonists (also referred to herein as an “IL-21/IL-21R agonists”), which increase the level, activity, and/or signal transduction of an IL-21 and/or an IL-21R, and/or an interaction between an IL-21 and an IL-21R, or antagonists of IL-21 and/or IL-21R (also referred to herein as an “IL-21/IL-21R antagonists”), which decrease the level, activity, and/or signal transduction of an IL-21 and/or an IL-21R, and/or an interaction between an IL-21 and an IL-21R, are disclosed herein. IL-21/IL-21R agonists can be used to increase the levels and/or activity of Treg cells and Foxp3, thus providing a means for treating, ameliorating, or preventing, e.g., inflammatory or autoimmune disorders, lymphopenia, graft-versus-host disease (GvHD), and transplant/graft rejection (e.g., disorders associated with reduced levels and/or activity of Treg cells and/or Foxp3, disorders associated with an enhanced immune response or disorders that would benefit from suppression of immunity). IL-21/IL-21R antagonists can be used to decrease the levels and/or activity of Treg cells and Foxp3, thus providing a means for treating, ameliorating, or preventing cancers, infectious disorders and HIV transcription (e.g., disorders associated with enhanced levels and/or activity of Treg cells and/or Foxp3, or disorders associated with a suppressed immune response, or disorders that would benefit from stimulation of immunity).

**[0063]** Applicants have shown that blockade of IL-21 in SJL/J mice before and after the induction of EAE enhances the influx of inflammatory cells into the central nervous system (CNS), as well as the severity of EAE. The blockade of IL-21 leads to proliferation of proteolipid peptide (PLP<sub>139-151</sub>)–autoreactive CD4<sup>+</sup> T cells, which are capable of causing severe EAE in adoptively transferred recipient mice.

Correspondingly, Tregs from mice in which IL-21 was blocked lose their capacity to prevent EAE induced by PLP<sub>139-151</sub>-reactive T cells. Notably, direct effects of IL-21 on Tregs were confirmed by studies of blockade of IL-21 in mice expressing a green fluorescent protein (GFP) “knocked” into a Foxp3 allele, in which a reduction of the number of Tregs and a downregulation of their frequency and expression of Foxp3 were observed.



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[0064] Applicants have also shown that IL-21R deficiency similarly increases the severity of EAE and enhances the influx of inflammatory cells into the CNS. Additionally, IL-21R deficiency is associated with a temporary expansion of Th1 and Th2 responses (particularly Th1), as shown by increased expression of IFN- $\gamma$ , IL-4, and IL-10 in CD4<sup>+</sup> and CD8<sup>+</sup> T cells of IL-21R<sup>-/-</sup> mice. IL-21R<sup>-/-</sup> mice also showed reduced numbers of splenic NK cells in the early stage of EAE and increased numbers of CNS NK cells during later stages of the disease. Taken together, these data suggest a role for the IL-21/IL-21R axis in the homeostasis of Tregs, Foxp3, and NK cells in immunity.

[0065] Accordingly, agents that agonize the activity, levels and/or signal transduction of IL-21/IL-21R and/or enhance the interaction of IL-21 with IL-21R can be used to decrease immune activity *in vivo*, *ex vivo*, and/or *in vitro*, e.g., for treating, ameliorating, or preventing inflammatory or autoimmune disorders (e.g., multiple sclerosis (MS), glomerulonephritis, transplant/graft rejection, psoriasis, atopic disorders, asthma, rheumatoid arthritis, IBD (e.g., Crohn's disease, ulcerative colitis), and systemic lupus erythematosus (SLE)). Agents that antagonize the activity, levels, and/or signal transduction of IL-21/IL-21R and/or suppress the interaction of IL-21 with IL-21R can be used to enhance immune activity *in vivo*, *ex vivo*, and/or *in vitro*, e.g., for treating, ameliorating, or preventing, e.g., cancers, infectious disorders, or HIV transcription.

#### Terms and Phrases

[0066] The phrase "interleukin-21 receptor" and the term "IL-21R" as used herein, refer to a class I cytokine family receptor, also known as NILR or Zalphall (WO 01/85792; Parrish-Novak et al. (2000) *supra*; Ozaki et al. (2000) *supra*), which binds to an IL-21 ligand. IL-21R is homologous to the shared  $\beta$  chain of the IL-2 and IL-15 receptors, and IL-4 $\alpha$  (Ozaki et al. (2000) *supra*). Upon ligand binding, IL-21R is capable of interacting with a common  $\gamma$  cytokine receptor chain ( $\gamma_c$ ) and inducing the phosphorylation of STAT1 and STAT3 (Asao et al. (2001) *supra*) or STAT5 (Ozaki et al. (2000) *supra*). IL-21R shows widespread lymphoid tissue

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distribution. The term "IL-21R" refers to a polypeptide (preferably of mammalian origin, e.g., murine or human IL-21R), or as context requires, a polynucleotide encoding such a polypeptide, that is capable of interacting with, e.g., binding to, an IL-21 (preferably an IL-21 of mammalian origin, e.g., murine or human IL-21) and having one of the following features: (i) an amino acid sequence of a naturally occurring mammalian IL-21R polypeptide or a fragment thereof, e.g., an amino acid sequence set forth in SEQ ID NO:2 (human - corresponding to GENBANK® Accession No. NP\_068570) or SEQ ID NO:4 (murine - corresponding to GENBANK® Acc. No. NP\_068687) or a fragment thereof; (ii) an amino acid sequence substantially homologous to, e.g., at least 85%, 90%, 95%, 98%, or 99% homologous to, an amino acid sequence set forth in SEQ ID NO:2 or SEQ ID NO:4 or a fragment thereof; (iii) an amino acid sequence that is encoded by a naturally occurring mammalian IL-21R nucleotide sequence or fragment thereof (e.g., SEQ ID NO:1 (human - corresponding to GENBANK Accession No. NM\_021798) or SEQ ID NO:3 (murine - corresponding to GENBANK® Acc. No. NM\_021887) or a fragment thereof); (iv) an amino acid sequence encoded by a nucleotide sequence which is substantially homologous to, e.g., at least 85%, 90%, 95%, 98%, 99% homologous to, a nucleotide sequence set forth in SEQ ID NO:1 or SEQ ID NO:3 or a fragment thereof; (v) an amino acid sequence encoded by a nucleotide sequence degenerate to a naturally occurring IL-21R nucleotide sequence or a fragment thereof, e.g., SEQ ID NO:1 or SEQ ID NO:3 or a fragment thereof; or (vi) a nucleotide sequence that hybridizes to one of the foregoing nucleotide sequences under stringent conditions, e.g., highly stringent conditions. In addition, other nonhuman and nonmammalian IL-21Rs are contemplated as useful in the disclosed methods.

[0067] As used herein, a "soluble IL-21R" is an IL-21R polypeptide incapable of being anchored to a cell membrane. Such soluble polypeptides include, for example, IL-21R polypeptides that lack a sufficient portion of their membrane-spanning domain or are modified such that the membrane-spanning domain is nonfunctional

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(e.g., a fragment of an IL-21R, comprising the extracellular domain of murine or human IL-21R, which includes an amino acid sequence from about amino acids 1-235, 1-236, 20-235, 20-236 of SEQ ID NO:2 (human), or from about amino acids 1-236, 20-236 of SEQ ID NO:4 (murine)). A soluble IL-21R polypeptide can additionally include, e.g., be fused to, a second moiety, e.g., a polypeptide (e.g., an immunoglobulin chain, a GST (i.e., glutathione S-transferase), Lex-A or MBP polypeptide sequence). For example, a fusion protein can include at least a fragment of an IL-21R polypeptide that is capable of binding IL-21 (e.g., a fragment of an IL-21R comprising the extracellular domain of murine or human IL-21R) fused to a second moiety, e.g., a polypeptide (e.g., an immunoglobulin chain, an Fc fragment, a heavy chain constant region of one of the various antibody isotypes, including: IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgD, and IgE). An exemplary fusion protein is set forth in SEQ ID NO:14.

**[0068]** The term “interleukin-21” or “IL-21” refers to a cytokine that shows sequence homology to IL-2, IL-4 and IL-15 (Parrish-Novak et al. (2000) *supra*) and which binds to an IL-21R. Cytokines share a common fold into a “four-helix-bundle” structure that is representative of the family. IL-21 is expressed primarily in activated CD4<sup>+</sup> T cells, and has been reported to have effects on NK, B and T cells (Parrish-Novak et al. (2000) *supra*; Kasaian et al. (2002) *supra*). IL-21 binds to IL-21R (also referred to herein as “MU-1,” “NILR,” and “zalpha11”). Upon IL-21 binding, activation of IL-21R leads to, e.g., STAT5 or STAT3 signaling (Ozaki et al. (2000) *supra*). The term “IL-21” refers to a polypeptide (preferably of mammalian origin, e.g., murine or human IL-21), or as context requires, a polynucleotide encoding such a polypeptide, that is capable of interacting with, e.g., binding to, an IL-21R (preferably of mammalian origin, e.g., murine or human IL-21R) and having one of the following features: (i) an amino acid sequence of a naturally occurring mammalian IL-21 or a fragment thereof, e.g., an amino acid sequence set forth in SEQ ID NO:9 (human) or SEQ ID NO:11 (murine) or a fragment thereof; (ii) an amino acid sequence substantially homologous to, e.g., at least 85%, 90%, 95%,

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98%, 99% homologous to, an amino acid sequence set forth in SEQ ID NO:9 or SEQ ID NO:11 or a fragment thereof; (iii) an amino acid sequence which is encoded by a naturally occurring mammalian IL-21 nucleotide sequence or a fragment thereof (e.g., SEQ ID NO:8 (human) or SEQ ID NO:10 (murine) or a fragment thereof); (iv) an amino acid sequence encoded by a nucleotide sequence which is substantially homologous to, e.g., at least 85%, 90%, 95%, 98%, 99% homologous to, a nucleotide sequence set forth in SEQ ID NO:8 or SEQ ID NO:10 or a fragment thereof; (v) an amino acid sequence encoded by a nucleotide sequence degenerate to a naturally occurring IL-21 nucleotide sequence or a fragment thereof, e.g., SEQ ID NO:8 or SEQ ID NO:10 or a fragment thereof; or (vi) a nucleotide sequence that hybridizes to one of the foregoing nucleotide sequences under stringent conditions, e.g., highly stringent conditions.

**[0069]** The phrases “IL-21 activity” and “IL-21R activity” and the like (e.g., “activity of IL-21” or “activity of IL-21R”) refer to one or more of the activities of the corresponding mature or immature IL-21 or IL-21R protein, including, but not limited to, (1) interacting with, e.g., binding to a ligand, e.g., an IL-21 polypeptide (e.g., a human IL-21 polypeptide) or interacting with, e.g., binding to a receptor, e.g., an IL-21R polypeptide (e.g., a human IL-21R polypeptide); (2) associating with or activating signal transduction (also called “signal transduction” or “signaling” herein, which phrases refer to the intracellular cascade occurring in response to a particular stimuli) and signal transduction molecules (e.g., common gamma chain, JAK1), and/or stimulating the phosphorylation and/or activation of STAT proteins, e.g., STAT5 and/or STAT3; and/or (3) modulating (e.g., stimulating or decreasing) the proliferation, differentiation, effector cell function, cytolytic activity, cytokine secretion, and/or survival of immune cells, e.g., T cells (CD8<sup>+</sup>CD4<sup>+</sup> T cells), NK cells, B cells, macrophages, regulatory T cells (Tregs) and megakaryocytes.

**[0070]** As used herein, a “therapeutically effective amount” of an IL-21/IL-21R agonist or antagonist refers to an amount that is effective, upon single or multiple dose administration to a subject, e.g., a human patient, to cure, treat, ameliorate, or

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prevent one or more symptoms of a disorder, e.g., a disorder as described herein, or in prolonging the survival of the subject beyond that expected in the absence of such treatment.

[0071] As used herein, “a prophylactically effective amount” of an IL-21/IL-21R agonist or antagonist refers to an amount that is effective, upon single or multiple dose administration to a subject, e.g., a human patient, to prevent or delay the occurrence of the onset or recurrence of a disorder, e.g., a disorder as described herein.

[0072] The term “in combination” in this context means that the agents are given substantially contemporaneously, either simultaneously or sequentially. If given sequentially, at the onset of administration of the second compound the first of the two compounds is preferably still detectable at an effective concentration at the site of treatment or in the subject.

[0073] As used herein, a “fusion protein” refers to a protein containing two or more operably associated moieties, e.g., linked moieties, e.g., protein moieties. Preferably, the moieties are covalently associated. The moieties can be directly associated or connected via a spacer or linker. As an example, an IL-21R extracellular domain may be fused to an Fc domain to produce a soluble IL-21R fusion protein.

[0074] As used herein, the term “antibody” refers to a protein comprising at least one, and optionally two, heavy (H) chain variable regions (abbreviated herein as VH), and at least one, and optionally two, light (L) chain variable regions (abbreviated herein as VL). The VH and VL regions can be further subdivided into regions of hypervariability, termed “complementarity determining regions” (CDRs), interspersed with regions that are more conserved, termed “framework regions” (FRs). The extent of the FRs and CDRs has been precisely defined (see, e.g., Kabat et al. (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242; and Chothia et al. (1987) *J. Mol. Biol.* 196:901-17, which are incorporated herein by reference in their entireties). Each VH and VL comprises three CDRs and four FRs,

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arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

**[0075]** The antibody can further include a heavy and light chain constant region, to thereby form a heavy and light immunoglobulin chain, respectively. In one embodiment, the antibody is a tetramer of two heavy immunoglobulin chains and two light immunoglobulin chains, wherein the heavy and light immunoglobulin chains are interconnected by, e.g., disulfide bonds. The heavy chain constant region is comprised of three domains: CH1, CH2, and CH3. The light chain constant region is comprised of one domain: CL. The variable region of the heavy and light chains contains a binding domain that interacts with an antigen. The constant region of an antibody typically mediates the binding of the antibody to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component (C1q) of the classical complement system.

**[0076]** As used herein, the term “immunoglobulin” refers to a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes. The recognized human immunoglobulin genes include the kappa, lambda, alpha (IgA1 and IgA2), gamma (IgG1, IgG2, IgG3, IgG4), delta, epsilon, and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Full-length immunoglobulin “light chains” (about 25 kDa or 214 amino acids) are encoded by a variable region gene at the NH<sub>2</sub>-terminus (about 110 amino acids) and a kappa or lambda constant region gene at the COOH-terminus. Full-length immunoglobulin “heavy chains” (about 50 kDa or 446 amino acids), are similarly encoded by a variable region gene (about 116 amino acids) and one of the other aforementioned constant region genes, e.g., gamma (encoding about 330 amino acids).

**[0077]** As used herein, “isotype” refers to the antibody class (e.g., IgM or IgG1) that is encoded by heavy chain constant region genes.

**[0078]** The term “antigen-binding fragment” of an antibody (or simply “antibody portion,” or “fragment”), as used herein, refers to one or more fragments of a full-length antibody that retain the ability to specifically bind to an antigen (e.g.,

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mammalian IL-21). Examples of binding fragments encompassed within the term “antigen-binding fragment” of an antibody include, but are not limited to: (i) an Fab fragment, a monovalent fragment consisting of the VL, VH, CL, and CH1 domains; (ii) an F(ab')<sub>2</sub> fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) an Fd fragment consisting of the VH and CH1 domains; (iv) an Fv or scFv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward et al. (1989) *Nature* 341:544-46), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR). Although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see, e.g., Bird et al. (1988) *Science* 242:423-26; and Huston et al. (1988) *Proc. Natl. Acad. Sci. U.S.A.* 85:5879-83). Such single chain antibodies are encompassed within the term “antigen-binding fragment” of an antibody. These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

**[0079]** Sequences similar or homologous (e.g., at least about 85% sequence identity) to the IL-21 and IL-21R sequences disclosed herein may also be used in the methods disclosed herein, e.g., as IL-21/IL-21R agonists or antagonists. In some embodiments, the sequence identity can be about 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or higher. Alternatively, substantial identity exists when the nucleic acid segments will hybridize under selective hybridization conditions (e.g., highly stringent hybridization conditions) to the complement of the strand. The nucleic acids may be present in whole cells, in a cell lysate, or in a partially purified or substantially pure form.

**[0080]** Calculations of “homology” or “sequence identity” between two sequences (the terms are used interchangeably herein) are performed as follows. The sequences

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are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and nonhomologous sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, 90%, or 100% of the length of the reference sequence. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

[0081] The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In one embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch algorithm ((1970) *J. Mol. Biol.* 48:444-53), which has been incorporated into the GAP program in the GCG software package (available at [www.gcg.com](http://www.gcg.com)) using either a BLOSUM 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package using an NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. A particular set of parameters (and the one that should be used if the practitioner is uncertain about what parameters should be applied to determine if a molecule is within a sequence identity or homology limitation of the



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invention) are a BLOSUM 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5. The percent identity between two amino acid or nucleotide sequences can also be determined using the algorithm of Meyers and Miller ((1989) *CABIOS* 4:11-17), which has been incorporated into the ALIGN program (version 2.0) using a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4.

[0082] As used herein, the term “hybridizes under stringent conditions” describes conditions for hybridization and washing. Stringent conditions are known to those skilled in the art and can be found in, e.g., *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. Aqueous and nonaqueous methods are described in this reference, and either can be used. An example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 50°C. In other embodiments of stringent hybridization conditions, the hybridization in 6X SSC at about 45°C can be followed by one or more washes in 0.2X SSC, 0.1% SDS at 55°C, 60°C, or 65°C. In another embodiment, highly stringent conditions (and the conditions that should be used if the practitioner is uncertain about what conditions should be applied to determine if a molecule is within a hybridization limitation of the invention) are 0.5M sodium phosphate, 7% SDS at 65°C, followed by one or more washes at 0.2X SSC, 1% SDS at 65°C. The disclosed IL-21 and IL-21R polynucleotides (IL-21/IL-21R polynucleotides) may be used as hybridization probes and primers to identify and isolate additional IL-21 polynucleotides and IL-21R polynucleotides that have sequences nearly identical to or similar to those encoding the disclosed IL-21/IL-21R polynucleotides, and which consequently may be used to produce additional IL-21/IL-21R agonists and antagonists. Hybridization methods for identifying and isolating nucleic acids include polymerase chain reaction (PCR), Southern hybridizations, *in situ* hybridization, and Northern hybridization, and are well known to those skilled in the

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art. Further disclosure regarding hybridization conditions and reactions is provided herein.

[0083] Hybridization reactions can be performed under conditions of different stringency. The stringency of a hybridization reaction includes the difficulty with which any two nucleic acid molecules will hybridize to one another. Preferably, each hybridizing polynucleotide hybridizes to its corresponding polynucleotide under reduced stringency conditions, more preferably stringent conditions, and most preferably highly stringent conditions. Examples of stringency conditions are shown in **Table 1** below: highly stringent conditions are those that are at least as stringent as, for example, conditions A-F; stringent conditions are at least as stringent as, for example, conditions G-L; and reduced stringency conditions are at least as stringent as, for example, conditions M-R.

**Table 1**

Stringency Condition	Poly-nucleotide Hybrid	Hybrid Length (bp) <sup>1</sup>	Hybridization Temperature and Buffer <sup>2</sup>	Wash Temperature and Buffer <sup>2</sup>
A	DNA:DNA	> 50	65°C; 1X SSC -or- 42°C; 1X SSC, 50% formamide	65°C; 0.3X SSC
B	DNA:DNA	<50	T <sub>B</sub> *; 1X SSC	T <sub>B</sub> *; 1X SSC
C	DNA:RNA	> 50	67°C; 1X SSC -or- 45°C; 1X SSC, 50% formamide	67°C; 0.3X SSC
D	DNA:RNA	<50	T <sub>D</sub> *; 1X SSC	T <sub>D</sub> *; 1X SSC
E	RNA:RNA	>50	70°C; 1X SSC -or- 50°C; 1X SSC, 50% formamide	70°C; 0.3X SSC
F	RNA:RNA	<50	T <sub>F</sub> *; 1X SSC	T <sub>F</sub> *; 1X SSC
G	DNA:DNA	>50	65°C; 4X SSC -or- 42°C; 4X SSC, 50% formamide	65°C; 1X SSC
H	DNA:DNA	<50	T <sub>H</sub> *; 4X SSC	T <sub>H</sub> *; 4X SSC
I	DNA:RNA	>50	67°C; 4X SSC -or- 45°C; 4X SSC, 50% formamide	67°C; 1X SSC
J	DNA:RNA	<50	T <sub>J</sub> *; 4X SSC	T <sub>J</sub> *; 4X SSC
K	RNA:RNA	>50	70°C; 4X SSC -or- 50°C; 4X SSC, 50% formamide	67°C; 1X SSC
L	RNA:RNA	<50	T <sub>L</sub> *; 2X SSC	T <sub>L</sub> *; 2X SSC
M	DNA:DNA	>50	50°C; 4X SSC -or- 40°C; 6X SSC,	50°C; 2X SSC

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			50% formamide	
N	DNA:DNA	<50	$T_N^*$ ; 6X SSC	$T_N^*$ ; 6X SSC
O	DNA:RNA	>50	55°C; 4X SSC -or- 42°C; 6X SSC, 50% formamide	55°C; 2X SSC
P	DNA:RNA	<50	$T_P^*$ ; 6X SSC	$T_P^*$ ; 6X SSC
Q	RNA:RNA	>50	60°C; 4X SSC -or- 45°C; 6X SSC, 50% formamide	60°C; 2X SSC
R	RNA:RNA	<50	$T_R^*$ ; 4X SSC	$T_R^*$ ; 4X SSC

<sup>1</sup> The hybrid length is that anticipated for the hybridized region(s) of the hybridizing polynucleotides. When hybridizing a polynucleotide to a target polynucleotide of unknown sequence, the hybrid length is assumed to be that of the hybridizing polynucleotide. When polynucleotides of known sequence are hybridized, the hybrid length can be determined by aligning the sequences of the polynucleotides and identifying the region or regions of optimal sequence complementarity.

<sup>2</sup> SSPE (1xSSPE is 0.15 M NaCl, 10 mM NaH<sub>2</sub>PO<sub>4</sub>, and 1.25 mM EDTA, pH 7.4) can be substituted for SSC (1xSSC is 0.15 M NaCl and 15 mM sodium citrate) in the hybridization and wash buffers; washes are performed for 15 min after hybridization is complete.

$T_B^* - T_R^*$ : The hybridization temperature for hybrids anticipated to be less than 50 base pairs in length should be 5-10°C less than the melting temperature ( $T_m$ ) of the hybrid, where  $T_m$  is determined according to the following equations. For hybrids less than 18 base pairs in length,  $T_m(^{\circ}\text{C}) = 2(\# \text{ of A} + \text{T bases}) + 4(\# \text{ of G} + \text{C bases})$ . For hybrids between 18 and 49 base pairs in length,  $T_m(^{\circ}\text{C}) = 81.5 + 16.6(\log_{10}\text{Na}^+) + 0.41(\% \text{G} + \text{C}) - (600/\text{N})$ , where N is the number of bases in the hybrid, and  $\text{Na}^+$  is the concentration of sodium ions in the hybridization buffer ( $\text{Na}^+$  for 1X SSC = 0.165 M).

Additional examples of stringency conditions for polynucleotide hybridization are provided in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Chs. 9, 11, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989), and Ausubel et al., eds., *Current Protocols in Molecular Biology*, Sects. 2.10 & 6.3-6.4, John Wiley & Sons, Inc. (1995), herein incorporated by reference.

[0084] IL-21/IL-21R polynucleotides also include allelic variants that encode polypeptides that are identical to or have significant similarity to the IL-21 and IL-21R polypeptides (IL-21/IL-21R polypeptides) encoded by the disclosed IL-21/IL-21R polynucleotides. Preferably, allelic variants have at least 90% sequence identity (more preferably, at least 95% identity; most preferably, at least 99% identity) with the disclosed polynucleotides.

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[0085] IL-21/IL-21R polynucleotides may also be used as hybridization probes and primers to identify and isolate additional useful IL-21 and IL-21R DNAs (e.g., for use as IL-21/IL-21R agonists and antagonists) having sequences encoding polypeptides homologous to the disclosed IL-21/IL-21R polynucleotides. These homologs are polynucleotides and polypeptides isolated from a different species than that of the disclosed polypeptides and polynucleotides, or within the same species, but with significant sequence similarity to the disclosed polynucleotides and polypeptides. Preferably, polynucleotide homologs have at least 50% sequence identity (more preferably, at least 75% identity; most preferably, at least 90% identity) with the disclosed polynucleotides, whereas polypeptide homologs have at least 30% sequence identity (more preferably, at least 45% identity; most preferably, at least 60% identity) with the disclosed polypeptides. Preferably, homologs of the disclosed polynucleotides and polypeptides are those isolated from mammalian species.

[0086] It is understood that the IL-21/IL-21R antagonists and agonists for use in the disclosed methods may have additional conservative or nonessential amino acid substitutions relative to naturally occurring IL-21/IL-21R polypeptides, which do not have a substantial effect on their functions. A “conservative amino acid substitution” is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine), and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine).

[0087] As used herein, “operably linked” means enzymatically or chemically ligated to form a covalent bond between the isolated polynucleotide of the invention and the

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expression control sequence, in such a way that the IL-21/IL-21R polypeptide is expressed by a host cell that has been transformed (transfected) with the ligated polynucleotide/expression control sequence.

[0088] The term “vector,” as used herein, is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a “plasmid,” which refers to a circular double-stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., nonepisomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as “recombinant expression vectors” (or simply “expression vectors”). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, “plasmid” and “vector” may be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

[0089] The term “regulatory sequence” is intended to include promoters, enhancers, and other expression control elements (e.g., polyadenylation signals) that control the transcription or translation of the antibody chain genes. Such regulatory sequences are described, for example, in Goeddel (1990) *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA. It will be appreciated by those skilled in the art that the design of the expression vector, including the selection of regulatory sequences may depend on factors that include the choice of

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the host cell to be transformed, the level of expression of protein desired, etc. Preferred regulatory sequences for mammalian host cell expression include viral elements that direct high levels of mRNA transcription or protein expression in mammalian cells, such as promoters and/or enhancers derived from FF-1a promoter and BGH poly A, cytomegalovirus (CMV) (such as the CMV promoter/enhancer), Simian Virus 40 (SV40) (such as the SV40 promoter/enhancer), adenovirus, (e.g., the adenovirus major late promoter (AdMLP)) and polyoma. For further description of viral regulatory elements, and sequences thereof, see, e.g., U.S. Patent Nos. 5,168,062; 4,510,245; and 4,968,615; the contents of all of which are hereby incorporated by reference herein.

**[0090]** The term “recombinant host cell” (or simply “host cell”), as used herein, is intended to refer to a cell into which a recombinant expression vector has been introduced. It should be understood that such terms are intended to refer not only to the particular subject cell but also to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term “host cell” as used herein.

**[0091]** The term “pharmaceutically acceptable carrier” means a nontoxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s). The characteristics of the carrier will depend on the route of administration.

**[0092]** As used herein, the term “therapeutically effective amount” means the total amount of each active component of the pharmaceutical composition or method that is sufficient to show a meaningful patient benefit, e.g., amelioration of symptoms of, healing of, or increase in rate of healing of such conditions. When applied to an individual active ingredient, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined (e.g., proportioned) amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

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#### IL-21/IL-21R Agonists and Antagonists

**[0093]** As used herein, an “IL-21/IL-21R antagonist” refers to an agent that decreases the level, activity, and/or signal transduction of an IL-21 and/or an IL-21R, and/or an interaction between an IL-21 and an IL-21R. Thus, the phrase “IL-21/IL-21R antagonist” includes both IL-21 antagonists and IL-21R antagonists. In one embodiment, the IL-21/IL-21R antagonist interacts with, e.g., binds to, an IL-21R polypeptide. In another embodiment, the IL-21/IL-21R antagonist interacts with, e.g., binds to, an IL-21 polypeptide. Antagonism using an IL-21/IL-21R antagonist may result in, e.g., inhibition, reduction, or a decrease in the level, activity, and/or signal transduction of an IL-21 and/or an IL-21R, and/or an interaction between an IL-21 and an IL-21R. IL-21/IL-21R antagonists include antagonistic IL-21/IL-21R polynucleotides (e.g., antisense, siRNA, aptamers); antagonistic IL-21/IL-21R polypeptides (e.g., fragments of IL-21/IL-21R, such as soluble IL-21Rs containing an extracellular ligand-binding domain, and fusion proteins thereof); antagonistic anti-IL-21/IL-21R antibodies or fragments thereof (including antibodies and antibody fragments that bind IL-21/IL-21R fragments); and antagonistic small molecules (e.g., siRNAs, aptamers, PNAs, and small organic molecules or compounds).

**[0094]** As used herein, an “IL-21/IL-21R agonist” refers to an agent that increases or enhances the level, activity, and/or signal transduction of an IL-21 and/or an IL-21R, and/or an interaction between an IL-21 and an IL-21R. Thus, the phrase “IL-21/IL-21R agonist” includes both IL-21 agonists and IL-21R agonists. In one embodiment, the IL-21/IL-21R agonist interacts with, e.g., binds to, an IL-21R polypeptide. In another embodiment, the IL-21/IL-21R agonist interacts with, e.g., binds to, an IL-21 polypeptide. Agonism using an IL-21/IL-21R agonist may result in, e.g., inducement, enhancement, or an increase in level, activity, and/or signal transduction of an IL-21 and/or an IL-21R, and/or an interaction between an IL-21 and an IL-21R. IL-21/IL-21R agonists include agonistic IL-21/IL-21R polynucleotides and fragments thereof; agonistic IL-21/IL-21R polypeptides and

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fragments thereof (including full-length and/or fragments of IL-21/IL-21R, and fusions thereof); agonistic anti-IL-21/IL-21R antibodies and fragments thereof; and agonistic small molecules (e.g., aptamers, PNAs, and small organic molecules or compounds).

#### Agonistic and Antagonistic IL-21/IL-21R Fusion Proteins

**[0095]** In one embodiment, an IL-21/IL-21R polypeptide or fragment thereof may be fused to a second moiety, e.g., an immunoglobulin or a fragment thereof (e.g., an Fc binding fragment thereof). For example, soluble forms of the IL-21R may be fused through “linker” sequences to the Fc portion of an immunoglobulin. Other fusion proteins, such as those with GST, Lex-A or MBP, may also be used. An exemplary fusion protein is set forth in SEQ ID NO:14, which contains an extracellular domain of human IL-21R fused to an Fc region.

**[0096]** IL-21/IL-21R fusion proteins may additionally include a linker sequence joining the IL-21 or IL-21R fragment to the second moiety. For example, the fusion protein can include a peptide linker, e.g., a peptide linker of about 4 to 20, more preferably 5 to 10, amino acids in length; in one embodiment, the peptide linker is 8 amino acids in length. Each of the amino acids in the peptide linker is selected from the group consisting of Gly, Ser, Asn, Thr, and Ala; in one embodiment, the peptide linker includes a Gly-Ser element. In other embodiments, the fusion protein includes a peptide linker and the peptide linker includes a sequence having the formula (Ser-Gly-Gly-Gly-Gly)<sub>y</sub> (see SEQ ID NO:25) wherein y is 1, 2, 3, 4, 5, 6, 7, or 8.

**[0097]** In other embodiments, additional amino acid sequences can be added to the N- or C-terminus of the fusion protein to facilitate expression, detection, and/or isolation or purification. For example, an IL-21/IL-21R fusion protein may be linked to one or more additional moieties, e.g., GST, His<sub>6</sub> tag, FLAG tag. For example, the fusion protein may additionally be linked to a GST fusion protein in which the fusion protein sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of the IL-21/IL-21R fusion protein.



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[0098] In another embodiment, the fusion protein includes a heterologous signal sequence (i.e., a polypeptide sequence that is not present in a polypeptide encoded by an IL-21/IL-21R nucleic acid) at its N-terminus. For example, the native IL-21R signal sequence can be removed and replaced with a signal sequence from another protein. In certain host cells (e.g., mammalian host cells), expression and/or secretion of IL-21/IL-21R can be increased through use of a heterologous signal sequence.

[0099] A chimeric or fusion protein of the invention can be produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different IL-21/IL-21R polypeptide sequences are ligated together in-frame in accordance with conventional techniques, e.g., by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers that give rise to complementary overhangs between two consecutive gene fragments that can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, e.g., Ausubel et al. (eds.) *Current Protocols in Molecular Biology*, John Wiley & Sons, 1992). Moreover, many expression vectors are commercially available that encode a fusion moiety (e.g., an Fc region of an immunoglobulin heavy chain). An IL-21/IL-21R-encoding polynucleotide can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the immunoglobulin protein. In some embodiments, IL-21/IL-21R fusion polypeptides exist as oligomers, such as dimers or trimers. The first polypeptide, and/or nucleic acids encoding the first polypeptide, can be constructed using methods known in the art.

[0100] In some embodiments, the IL-21/IL-21R polypeptide moiety is provided as a variant IL-21/IL-21R polypeptide having a mutation in the naturally occurring

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IL-21/IL-21R sequence (wild type) that results in higher activity, e.g., higher affinity (relative to the nonmutated sequence) binding of an IL-21R polypeptide to an IL-21.

[0101] In some embodiments, the IL-21/IL-21R polypeptide or fragment thereof is provided as a variant IL-21/IL-21R polypeptide having mutations in the naturally occurring IL-21/IL-21R sequence (wild type) that results in an IL-21/IL-21R sequence more resistant to proteolysis (relative to the nonmutated sequence). In some embodiments, the first polypeptide includes a full-length IL-21R polypeptide. Alternatively, the first polypeptide comprises a less than full-length IL-21R polypeptide.

[0102] As a nonlimiting example, one signal peptide that can be included in the fusion protein is MPLLLLLLLLLPSPLHP (SEQ ID NO:6). If desired, one or more amino acids can additionally be inserted between the first polypeptide moiety comprising the IL-21/IL-21R moiety and the second polypeptide moiety.

[0103] The second polypeptide is preferably soluble. In some embodiments, the second polypeptide enhances the half-life (e.g., the serum half-life) of the linked polypeptide. In some embodiments, the second polypeptide includes a sequence that facilitates association of the fusion polypeptide with a second IL-21/IL-21R polypeptide. In preferred embodiments, the second polypeptide includes at least a region of an immunoglobulin polypeptide. Immunoglobulin fusions polypeptides are known in the art and are described in, e.g., U.S. Patent Nos. 5,516,964; 5,225,538; 5,428,130; 5,514,582; 5,714,147; and 5,455,165; the contents of all of which are hereby incorporated by reference herein.

[0104] In some embodiments, the second polypeptide comprises a full-length immunoglobulin polypeptide. Alternatively, the second polypeptide comprises a less than full-length immunoglobulin polypeptide, e.g., a heavy chain, light chain, Fab, Fab<sub>2</sub>, Fv, or Fc. Preferably, the second polypeptide includes the heavy chain of an immunoglobulin polypeptide. More preferably, the second polypeptide includes the Fc region of an immunoglobulin polypeptide.

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**[0105]** In another aspect of the invention, the second polypeptide has less effector function than the effector function of an Fc region of a wild-type immunoglobulin heavy chain. Fc effector function includes for example, Fc receptor binding, complement fixation, and T cell-depleting activity (see, e.g., U.S. Patent No. 6,136,310). Methods for assaying T cell-depleting activity, Fc effector function, and antibody stability are known in the art. In one embodiment, the second polypeptide has low or no affinity for the Fc receptor. In an alternative embodiment, the second polypeptide has low or no affinity for complement protein C1q. A preferred second polypeptide sequence includes the amino acid sequence of SEQ ID NO:7.

**[0106]** Exemplary IL-21R antagonistic fusion proteins that can be used in the methods of the invention are shown in U.S. Published Patent Application No. 2006/0039902. In one embodiment, an IL-21R fusion protein for use in the disclosed methods has the amino acid sequence of SEQ ID NO:14.

#### Agonistic and Antagonistic Anti-IL-21/IL-21R Antibodies

**[0107]** In other embodiments, the IL-21/IL-21R antagonists or agonists are antibodies or antigen-binding fragments thereof that bind to IL-21 or IL-21R, preferably, mammalian (e.g., human or murine) IL-21 or IL-21R, and antagonize their activity (e.g., inhibit, reduce, or suppress IL-21 or IL-21R activity) or agonize their activity (e.g., enhance, stimulate, or increase IL-21 or IL-21R activity).

**[0108]** IL-21/IL-21R polypeptides of the invention may also be used to immunize animals to obtain polyclonal and monoclonal antibodies that specifically react with the IL-21/IL-21R polypeptides and that can inhibit binding of IL-21 ligands to IL-21Rs. Such antibodies may be obtained using the entire IL-21 or IL-21R as an immunogen, or by using fragments of IL-21 or IL-21R. Smaller fragments of the IL-21 or IL-21R can also be used to immunize animals. The peptide immunogens additionally may contain a cysteine residue at the C-terminus and are conjugated to a hapten such as keyhole limpet hemocyanin (KLH). Additional peptide immunogens may be generated by replacing tyrosine residues with sulfated tyrosine residues. Methods for synthesizing such peptides are well known in the art.

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[0109] Neutralizing or nonneutralizing antibodies (preferably monoclonal antibodies) binding to IL-21/IL-21R polypeptides may also be useful in the treatment of conditions or disorders described herein. For example, such neutralizing monoclonal antibodies may be capable of blocking IL-21 ligand binding to the IL-21R chain.

[0110] Human monoclonal antibodies (mAbs) directed against IL-21/IL-21R polypeptides or fragments thereof can be generated using transgenic mice carrying the human immunoglobulin genes rather than the mouse system. Splenocytes from these transgenic mice immunized with the antigen of interest are used to produce hybridomas that secrete human mAbs with specific affinities for epitopes from a human protein (see, e.g., Wood et al., International Publication WO 91/00906; Kucherlapati et al., International Publication WO 91/10741; Lonberg et al., International Publication WO 92/03918; Kay et al., International Publication WO 92/03917; Lonberg et al. (1994) *Nature* 368:856-59; Green et al. (1994) *Nat. Genet.* 7:13-21; Morrison et al. (1994) *Proc. Natl. Acad. Sci. U.S.A.* 81:6851-55; Bruggeman et al. (1993) *Year Immunol.* 7:33-40; Tuaillon et al. (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:3720-24; Bruggeman et al. (1991) *Eur. J. Immunol.* 21:1323-26; the contents of all of which are hereby incorporated by reference herein).

[0111] Monoclonal antibodies can also be generated by other methods known to those skilled in the art of recombinant DNA technology. An alternative method, referred to as the "combinatorial antibody display" method, has been developed to identify and isolate antibody fragments having a particular antigen specificity, and can be utilized to produce monoclonal antibodies; this method is well known in the art. After immunizing an animal with an immunogen, the antibody repertoire of the resulting B cell pool is cloned. Methods are generally known for obtaining the DNA sequence of the variable regions of a diverse population of immunoglobulin molecules by using a mixture of oligomer primers and PCR. For instance, mixed oligonucleotide primers corresponding to the 5' leader (signal peptide) sequences and/or framework 1 (FR1) sequences, as well as primer to a conserved 3' constant

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region primer, can be used for PCR amplification of the heavy and light chain variable regions from a number of murine antibodies (Larrick et al. (1991) *Biotechniques* 11:152-56). A similar strategy can also be used to amplify human heavy and light chain variable regions from human antibodies (Larrick et al. (1991) *Methods: Companion to Methods in Enzymology* 2:106-10).

[0112] Chimeric antibodies, including chimeric immunoglobulin chains, can be produced by recombinant DNA techniques known in the art. For example, a gene encoding the Fc constant region of a murine (or other species) monoclonal antibody molecule is digested with restriction enzymes to remove the region encoding the murine Fc, and the equivalent portion of a gene encoding a human Fc constant region is substituted (see, e.g., Robinson et al., International Patent Publication PCT/US86/02269; Akira et al., European Patent Application 184,187; Taniguchi, European Patent Application 171,496; Morrison et al., European Patent Application 173,494; Neuberger et al., International Publication WO 86/01533; Cabilly et al., U.S. Patent No. 4,816,567; Cabilly et al., European Patent Application 125,023; Better et al. (1988) *Science* 240:1041-43; Liu et al. (1987) *Proc. Natl. Acad. Sci. U.S.A.* 84:3439-43; Liu et al. (1987) *J. Immunol.* 139:3521-26; Sun et al. (1987) *Proc. Natl. Acad. Sci. U.S.A.* 84:214-18; Nishimura et al. (1987) *Canc. Res.* 47:999-1005; Wood et al. (1985) *Nature* 314:446-49; Shaw et al. (1988) *J. Natl. Cancer Inst.* 80:1553-59; the contents of all of which are hereby incorporated by reference herein).

[0113] An antibody or an immunoglobulin chain can be humanized by methods known in the art. Humanized antibodies, including humanized immunoglobulin chains, can be generated by replacing sequences of the Fv variable region that are not directly involved in antigen binding with equivalent sequences from human Fv variable regions. General methods for generating humanized antibodies are provided by Morrison (1985) *Science* 229:1202-07; Oi et al. (1986) *BioTechniques* 4:214; and Queen et al., U.S. Patent Nos. 5,585,089; 5,693,761; and 5,693,762, the contents of all of which are hereby incorporated by reference herein. Those methods

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include isolating, manipulating, and expressing the nucleic acid sequences that encode all or part of immunoglobulin Fv variable regions from at least one of a heavy or light chain. Sources of such nucleic acids are well known to those skilled in the art and, for example, may be obtained from a hybridoma producing an antibody against a predetermined target. The recombinant DNA encoding the humanized antibody, or fragment thereof, then can be cloned into an appropriate expression vector.

[0114] Humanized or CDR-grafted antibody molecules or immunoglobulins can be produced by CDR grafting or CDR substitution, wherein one, two, or all CDRs of an immunoglobulin chain can be replaced (see, e.g., U.S. Patent No. 5,225,539; Jones et al. (1986) *Nature* 321(6069):522-25; Verhoeyen et al. (1988) *Science* 239(4847):1534-36; Beidler et al. (1988) *J. Immunol.* 141:4053-60; Winter, U.S. Patent No. 5,225,539; the contents of all of which are hereby incorporated by reference herein). Winter describes a CDR-grafting method that may be used to prepare the humanized antibodies of the present invention (G.B. Patent No. 8707252; Winter, U.S. Patent No. 5,225,539, the contents of which are hereby incorporated by reference herein). All of the CDRs of a particular human antibody may be replaced with at least a portion of a nonhuman CDR, or only some of the CDRs may be replaced with nonhuman CDRs. It is only necessary to replace the number of CDRs required for binding of the humanized antibody to a predetermined antigen.

[0115] Human antibodies to IL-21/IL-21R polypeptides or fragments thereof may additionally be produced using transgenic nonhuman animals that are modified to produce fully human antibodies rather than the animal's endogenous antibodies in response to challenge by an antigen. See, e.g., PCT publication WO 94/02602. The endogenous genes encoding the heavy and light immunoglobulin chains in the nonhuman host are incapacitated, and active loci encoding human heavy and light chain immunoglobulins are inserted into the host's genome. The human genes are incorporated, for example, using yeast artificial chromosomes containing the requisite human DNA segments. An animal which provides all the desired

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modifications is then obtained as progeny by crossbreeding intermediate transgenic animals containing fewer than the full complement of the modifications. One embodiment of such a nonhuman animal is a mouse, and is termed the XENOMOUSE™ as disclosed in PCT publications WO 96/33735 and WO 96/34096.

This animal produces B cells that secrete fully human immunoglobulins. The antibodies can be obtained directly from the animal after immunization with an immunogen of interest, as, for example, a preparation of a polyclonal antibody, or alternatively from immortalized B cells derived from the animal, such as hybridomas producing monoclonal antibodies. Additionally, the genes encoding the immunoglobulins with human variable regions can be recovered and expressed to obtain the antibodies directly, or can be further modified to obtain analogs of antibodies such as, for example, single chain Fv molecules.

[0116] Monoclonal, chimeric, and humanized antibodies that have been modified by, e.g., deleting, adding, or substituting other portions of the antibody, e.g., the constant region, are also within the scope of the invention. For example, an antibody can be modified by: (i) deleting the constant region; (ii) replacing the constant region with another constant region, e.g., a constant region meant to increase half-life, stability, or affinity of the antibody, or a constant region from another species or antibody class; and/or (iii) modifying one or more amino acids in the constant region to alter, for example, the number of glycosylation sites, effector cell function, Fc receptor (FcR) binding, complement fixation, etc.

[0117] Methods for altering an antibody constant region are known in the art. Antibodies with altered function, e.g., altered affinity for an effector ligand, such as FcR on a cell, or the C1 component of complement, can be produced by replacing at least one amino acid residue in the constant portion of the antibody with a different residue (see, e.g., E.P. Patent 388,151 A1; U.S. Patent 5,624,821; and U.S. Patent 5,648,260, the contents of all of which are hereby incorporated by reference herein).

Similar types of alterations can be described that, if applied to the murine or other species' immunoglobulin, would reduce or eliminate these functions.

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**[0118]** For example, it is possible to alter the affinity of an Fc region of an antibody (e.g., an IgG, such as a human IgG) for an FcR (e.g., Fc gamma R1), or for C1q binding by replacing the specified residue(s) with a residue(s) having an appropriate functionality on its side chain, or by introducing a charged functional group, such as glutamate or aspartate, or an aromatic nonpolar residue, such as phenylalanine, tyrosine, tryptophan, or alanine (see, e.g., U.S. Patent 5,624,821).

**[0119]** In addition to antibodies for use in the instant invention, other molecules may also be employed to modulate the activity of IL-21 and/or IL-21R. Such molecules include small modular immunopharmaceutical (SMIP™) drugs (Trubion Pharmaceuticals, Seattle, WA). SMIPs are single-chain polypeptides composed of a binding domain for a cognate structure such as an antigen, a counter receptor or the like, a hinge-region polypeptide having either one or no cysteine residues, and immunoglobulin CH2 and CH3 domains (see also [www.trubion.com](http://www.trubion.com)). SMIPs and their uses and applications are disclosed in, e.g., U.S. Published Patent Appln. Nos. 2003/0118592; 2003/0133939; 2004/0058445; 2005/0136049; 2005/0175614; 2005/0180970; 2005/0186216; 2005/0202012; 2005/0202023; 2005/0202028; 2005/0202534; and 2005/0238646, and related patent family members thereof, all of which are hereby incorporated by reference herein in their entireties.

#### Agonistic and Antagonistic IL-21/IL-21R Polynucleotides and Polypeptides

**[0120]** An IL-21R for use in the disclosed methods is of mammalian, preferably, human origin. The nucleotide sequence and the predicted amino acid sequence of human IL-21R are shown in SEQ ID NO:1 and SEQ ID NO:2, respectively.

Analysis of the human IL-21R amino acid sequence (SEQ ID NO:2) reveals the following structural features: a leader sequence (about amino acids 1-19 of SEQ ID NO:2); WSXWS motif (SEQ ID NO:15, corresponding to about amino acids 213-217 of SEQ ID NO:2); transmembrane domain (about amino acids 236-252 of SEQ ID NO:2); an extracellular domain from about amino acids 1-235 of SEQ ID NO:2; and an intracellular domain from about 253-538 of SEQ ID NO:2. The



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mature human IL-21R is believed to have the sequence of amino acids 20-538 of SEQ ID NO:2.

[0121] The human IL-21R cDNA was deposited with the American Type Culture Collection on March 10, 1998, as accession number ATCC 98687. Additional IL-21R sequences (polynucleotide and polypeptide) are known in the art and include mouse IL-21R polynucleotide (SEQ ID NO:3) and mouse IL-21R polypeptide (SEQ ID NO:4).

[0122] Any form of IL-21R polypeptides or polynucleotides of less than full length (i.e., fragments) can be used in the methods and compositions of the present invention, provided that such a fragment retains the ability to bind to an IL-21 polypeptide or the ability to encode a protein that retains the ability to bind to an IL-21 polypeptide. IL-21R fragments, e.g., the extracellular domain of an IL-21R (such as may be used in producing a soluble IL-21R), can be produced by expressing a corresponding fragment of the polynucleotide encoding the full-length IL-21R protein in a host cell. Modified polynucleotides as described above may be made by standard molecular biology techniques, including construction of appropriate desired deletion mutants, site-directed mutagenesis methods, or polymerase chain reaction.

[0123] Amino acid sequences of IL-21 polypeptides are publicly known. For example, a nucleotide sequence and amino acid sequence of a human IL-21 is available at GENBANK<sup>®</sup> Acc. Nos. BC069124 and AAH69124.1, respectively.

A human IL-21 nucleotide sequence is presented in SEQ ID NO:8. The amino acid sequence of the disclosed human IL-21 polypeptide is presented in SEQ ID NO:9.

[0124] The mouse IL-21 nucleotide sequence, which is set forth as SEQ ID NO:10, is available at GENBANK<sup>®</sup> Acc. No. NM\_021782.2, and the mouse IL-21 polypeptide sequence, which is set forth as SEQ ID NO:11, is available at GENBANK<sup>®</sup> Acc. No. NP\_068554. The rat IL-21 nucleotide sequence, which is set forth as SEQ ID NO:12, is available at GENBANK<sup>®</sup> Acc. No. DQ387062, and the rat IL-21 polypeptide sequence, which is set forth as SEQ ID NO:13, is available at GENBANK<sup>®</sup> Acc. No. ABD52001.

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[0125] The methods disclosed herein also encompass the use of nucleic acids that hybridize to the mouse, rat and/or human IL-21 and IL-21R nucleotide sequences set forth in SEQ ID NOs:1, 3, 8, 10 and 12.

[0126] IL-21/IL-21R polynucleotides (preferably mammalian IL-21/IL-21R polynucleotides) may be operably linked to an expression control sequence, such as the pMT2 or pED expression vectors disclosed in Kaufman et al. (1991) *Nucleic Acids Res.* 19:4485-90, in order to produce the IL-21/IL-21R polypeptides recombinantly. Many suitable expression control sequences are known in the art. General methods of expressing recombinant proteins are also known and are exemplified in, e.g., Kaufman (1990) *Methods in Enzymology* 185:537-66.

[0127] Recombinant expression vectors for expressing IL-21/IL-21R polypeptides and fragments thereof may carry additional sequences, such as sequences that regulate replication of the vector in host cells (e.g., origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see, e.g., U.S. Patents Nos. 4,399,216; 4,634,665; and 5,179,017). For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin, or methotrexate, on a host cell into which the vector has been introduced. Preferred selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in *dhfr*<sup>-</sup> host cells with methotrexate selection/amplification) and the *neo* gene (for G418 selection).

[0128] A number of types of cells may act as suitable host cells for expression of an IL-21/IL-21R polypeptide(s), or fragments or fusions thereof. Any cell type capable of expressing functional IL-21 and IL-21R protein may be used. Suitable mammalian host cells include, for example, monkey COS cells, Chinese hamster ovary (CHO) cells, human kidney 293 cells, human epidermal A431 cells, human Colo205 cells, 3T3 cells, CV-1 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from *in vitro* culture of primary tissue, primary explants, HeLa cells, mouse L cells, BHK, HL-60, U937, HaK, Rat2, BaF3, 32D, FDCP-1, PC12, M1x, or C2C12 cells.

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[0129] An IL-21/IL-21R polypeptide, or fragment or fusion thereof, may also be produced by operably linking an IL-21 or IL-21R polynucleotide to suitable control sequences in one or more insect expression vectors, and employing an insect expression system. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, e.g., Invitrogen, San Diego, CA (e.g., the MAXBAC<sup>®</sup> kit), and such methods are well known in the art, as described in Summers and Smith (1987) *Texas Agricultural Experiment Station Bulletin* No. 1555, incorporated by reference herein in its entirety. Soluble forms of IL-21/IL-21R polypeptides may also be produced in insect cells using appropriate isolated polynucleotides as described above.

[0130] Alternatively, IL-21/IL-21R polypeptides (or fragments or fusions thereof) may be produced in lower eukaryotes such as yeast or in prokaryotes such as bacteria. Suitable yeast strains include *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Kluyveromyces* strains, *Candida*, or any yeast strain capable of expressing heterologous proteins. Suitable bacterial strains include *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhimurium*, or any bacterial strain capable of expressing heterologous proteins.

[0131] Expression in bacteria may result in formation of inclusion bodies incorporating the recombinant protein. Thus, refolding of the recombinant protein may be required in order to produce active, or more active, material. Several methods for obtaining correctly folded heterologous proteins from bacterial inclusion bodies are known in the art. These methods generally involve solubilizing the protein from the inclusion bodies, then denaturing the protein completely using a chaotropic agent. When cysteine residues are present in the primary amino acid sequence of the protein, it is often necessary to accomplish the refolding in an environment that allows correct formation of disulfide bonds (a redox system). General methods of refolding are disclosed in Kohno (1990) *Meth. Enzym.* 185:187-95; other appropriate methods are described in E.P. 0433225 and U.S. Patent No. 5,399,677.

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[0132] An IL-21/IL-21R polypeptide or a fragment or fusion thereof may also be expressed as a product of transgenic animals, e.g., as a component of the milk of transgenic cows, goats, pigs, or sheep that are characterized by somatic or germ cells containing a polynucleotide sequence encoding the IL-21 or IL-21R protein or fragment, or a fusion protein thereof.

[0133] An IL-21/IL-21R polypeptide or a fragment or fusion thereof may be prepared by growing culture-transformed host cells under culture conditions necessary to express the desired protein. The resulting expressed protein may then be purified from the culture medium or cell extracts. Soluble forms of IL-21/IL-21R polypeptides, or fragments or fusions thereof, can be purified from conditioned media. Membrane-bound forms of IL-21 or IL-21R polypeptides or fusion proteins thereof can be purified by preparing a total membrane fraction from the expressing cell and extracting the membranes with a nonionic detergent such as TRITON® X-100.

[0134] An IL-21/IL-21R polypeptide or a fragment or fusion thereof can be purified using methods known to those skilled in the art. For example, an IL-21/IL-21R polypeptide or a fragment or fusion thereof can be concentrated using a commercially available protein concentration filter, for example, an AMICON® or PELLICON® ultrafiltration unit (Millipore, Billerica, MA). Following the concentration step, the concentrate can be applied to a purification matrix such as a gel-filtration medium. Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) or polyethyleneimine (PEI) groups. The matrices can be acrylamide, agarose, dextran, cellulose, or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. Sulfopropyl groups are preferred (e.g., S-SEPHAROSE® columns). The purification of an IL-21/IL-21R polypeptide or a fragment or fusion thereof from culture supernatant may also include one or more column steps over such affinity resins as

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concanavalin A-agarose, heparin-TOYOPEARL<sup>®</sup>, or Cibacron blue 3GA SEPHAROSE<sup>®</sup>; or by hydrophobic interaction chromatography using such resins as phenyl ether, butyl ether, or propyl ether; or by immunoaffinity chromatography. One or more reverse-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify the IL-21/IL-21R polypeptide or a fragment or fusion thereof. Affinity columns including antibodies to the IL-21/IL-21R polypeptide or a fragment thereof can also be used in purification in accordance with known methods. Some or all of the foregoing purification steps, in various combinations or with other known methods, can also be employed to provide a substantially purified isolated recombinant protein. Preferably, the isolated IL-21/IL-21R polypeptide or fragment or fusion thereof is purified so that it is substantially free of other mammalian proteins.

[0135] IL-21/IL-21R polypeptides or fragments or fusions thereof of the invention may also be used to screen for agents that are capable of binding to IL-21/IL-21R. Binding assays involving a desired binding protein, immobilized or not, are well known in the art and can be used for this purpose with an IL-21/IL-21R. Purified cell-based or protein-based (cell-free) screening assays may be used to identify such agents. For example, an IL-21 or IL-21R polypeptide may be immobilized in purified form on a carrier, and binding of potential ligands to purified IL-21 or IL-21R may be measured.

[0136] Altered expression of IL-21 or IL-21R may be achieved in a cell or organism through the use of various antagonistic (e.g., inhibitory) polynucleotides, such as antisense polynucleotides, siRNAs, and ribozymes that bind and/or cleave the mRNA transcribed from IL-21/IL-21R genes (see, e.g., Galderisi et al. (1999) *J. Cell Physiol.* 181:251-57; Sioud (2001) *Curr. Mol. Med.* 1:575-88). Antagonistic IL-21/IL-21R polynucleotides may also consist of aptamers, polynucleotides that bind to and regulate protein activity, e.g., the activity of human IL-21 or IL-21R.

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Aptamers are described in the literature (see, e.g., Nimjee et al. (2005) *Annu. Rev. Med.* 56:555-83; Patel (1997) *Curr. Opin. Chem. Biol.* 1:32-46).

[0137] Antagonistic IL-21/IL-21R polynucleotides also include triplex-forming oligonucleotides (TFOs) that bind in the major groove of duplex DNA with high specificity and affinity (Knauert and Glazer (2001) *Hum. Mol. Genet.* 10:2243-51). Expression of IL-21 and IL-21R can be inhibited by targeting TFOs complementary to the regulatory regions of the genes (i.e., the promoter and/or enhancer sequences) to form triple-helical structures that prevent transcription of the genes.

[0138] Antagonistic IL-21/IL-21R polynucleotides include short interfering RNA (siRNA) molecules useful for RNA interference (RNAi) (e.g., Bass (2001) *Nature* 411:428-29). siRNAs are small double-stranded RNAs (dsRNAs) that optionally include overhangs. For example, the duplex region of an siRNA is about 18 to 25 nucleotides in length, e.g., about 19, 20, 21, 22, 23, or 24 nucleotides in length. Typically the siRNA sequences are exactly complementary to the target mRNA. Such dsRNAs and siRNAs in particular can be used to silence gene expression in mammalian cells (e.g., human cells). See, e.g., Clemens et al. (2000) *Proc. Natl. Acad. Sci. USA* 97:6499-503; Billy et al. (2001) *Proc. Natl. Acad. Sci. USA* 98:14428-33; Elbashir et al. (2001) *Nature* 411(6836):494-98; Yang et al. (2002) *Proc. Natl. Acad. Sci. USA* 99:9942-47; U.S. Published Application Nos. 2003/0166282; 2003/0143204; 2004/0038278; and 2003/0224432. These siRNA molecules may be generated by a variety of methods that are well known in the art (Fire et al., U.S. Patent No. 6,506,559; Yu et al. (2002) *Proc. Natl. Acad. Sci. USA* 99:6047-52; Elbashir et al. (2001) *Nature* 411:494-98; Yu et al., *supra*; Sui et al. (2002) *Proc. Natl. Acad. Sci. USA* 99:5515-20; Paddison et al. (2002) *Proc. Natl. Acad. Sci. USA* 99:1443-48; Arts et al. (2003) *Genome Res.* 13:2325-32). The siRNA molecules targeted to IL-21/IL-21R polynucleotides can also be designed based on criteria well known in the art (e.g., Elbashir et al. (2001) *EMBO J.* 20:6877-88).

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[0139] Altered expression of IL-21 or IL-21R may also be achieved with PNAs (peptide nucleic acids) and/or aptamers. PNAs are synthetic homologs of nucleic acids in which the phosphate-sugar polynucleotide backbone is replaced by a flexible pseudo-peptide polymer to which the nucleobases are linked. This structure gives PNAs the capacity to hybridize with high affinity and specificity to complementary sequences of DNA and RNA, and also confers remarkable resistance to DNAses and proteinases. These unique features of PNAs make them apt to agonize or antagonize an IL-21/IL-21R pathway. PNAs and their synthesis are described in the literature (see, e.g., Ray and Norden (2000) *FASEB J.* 14:1041-60). Aptamers are oligonucleic acids (DNA or RNA) or peptide molecules that bind specific target molecules. They are usually created by selecting from a large random sequence pool, but also occur naturally in riboswitches (part of an mRNA molecule that can directly bind a small target molecule, thereby affecting the activity of a gene). Aptamers and their synthesis are also described in the literature (see, e.g., Jayasena et al. (1999) *Clin. Chem.* 45:1628-50).

#### Small Molecules

[0140] Modulating (e.g., increasing or decreasing) the levels, activity and/or signaling of IL-21 and/or IL-21R, and/or the interaction of an IL-21 with an IL-21R, in an organism may also be achieved through the use of agonistic and antagonistic small molecules (usually organic small molecules). Novel agonistic and antagonistic small molecules may be identified by the screening methods described herein and may be used in the treatment, amelioration, and/or prevention methods of the present invention described herein.

[0141] The term small molecule refers to compounds that are not macromolecules (see, e.g., Karp (2000) *Bioinformatics Ontology* 16:269-85; Verkman (2004) *AJP-Cell Physiol.* 286:465-74). Thus, small molecules are often considered those compounds that are, e.g., less than one thousand daltons (e.g., Voet and Voet, *Biochemistry*, 2<sup>nd</sup> ed., ed. N. Rose, Wiley and Sons, New York, 14 (1995)). For example, Davis et al. ((2005) *Proc. Natl. Acad. Sci. USA* 102:5981-86) use the

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phrase small molecule to indicate folates, methotrexate, and neuropeptides, whereas Halpin and Harbury ((2004) *PLoS Biology* 2:1022-30) use the phrase to indicate small molecule gene products, e.g., DNAs, RNAs, and peptides. Examples of small molecules include, but are not limited to, cholesterol, neurotransmitters, aptamers, and siRNAs. In addition, other small molecules include, but are not limited to, various synthetic chemicals listed in numerous commercially available small molecule databases, e.g., FCD (Fine Chemicals Database), SMID (Small Molecule Interaction Database), ChEBI (Chemical Entities of Biological Interest), and CSD (Cambridge Structural Database) (see, e.g., Alfarano et al. (2005) *Nuc. Acids Res. Database Issue* 33:D416-24).

#### Pharmaceutical Compositions

[0142] An IL-21/IL-21R agonist and/or antagonist may be used as a pharmaceutical composition when combined with a pharmaceutically acceptable carrier. Such a composition may contain, in addition to the IL-21/IL-21R agonist or antagonist and carrier, various diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art.

[0143] The pharmaceutical composition of the invention may be in the form of a liposome in which an IL-21/IL-21R agonist(s) or antagonist(s) is combined with, in addition to other pharmaceutically acceptable carriers, amphipathic agents such as lipids that exist in aggregated form as micelles, insoluble monolayers, liquid crystals, or lamellar layers that are in aqueous solution. Suitable lipids for liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfatides, lysolecithin, phospholipids, saponin, bile acids, and the like. Preparation of such liposomal formulations is within the level of skill in the art, as disclosed, e.g., in U.S. Patent Nos. 4,235,871; 4,501,728; 4,837,028; and 4,737,323, all of which are incorporated herein by reference in their entireties.

[0144] In practicing the methods of treatment or other uses of the present invention, a therapeutically effective amount of an IL-21/IL-21R agonist or antagonist is administered to a subject, e.g., mammal (e.g., a human). An IL-21/IL-21R agonist(s)



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or antagonist(s) may be administered in accordance with the method of the invention either alone or in combination with other therapies as described in more detail herein. When coadministered with one or more agents, an IL-21/IL-21R agonist or antagonist may be administered either simultaneously with the second agent, or sequentially. If administered sequentially, the attending physician will decide on the appropriate sequence of administering the IL-21/IL-21R agonist(s) or antagonist(s) in combination with other agents.

[0145] Administration of an IL-21/IL-21R agonist or antagonist used in a pharmaceutical composition of, or to practice the method of, the present invention can be carried out in a variety of conventional ways, such as, but not limited to, oral ingestion, inhalation, or cutaneous, subcutaneous, or intravenous injection. In some embodiments, intravenous administration to the patient is preferred.

[0146] When a therapeutically effective amount of an IL-21/IL-21R agonist or antagonist is administered orally, the binding agent will be in the form of a tablet, capsule, powder, solution, or elixir. When administered in tablet form, the pharmaceutical composition of the invention may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, or powder contains from about 5 to 95% binding agent, and preferably from about 25 to 90% binding agent. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as mineral oil, soybean oil, peanut oil, or sesame oil (taking into consideration the allergic responses of some patients), or synthetic oils, may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or another saccharide solution, or glycols such as ethylene glycol, propylene glycol, or polyethylene glycol. When administered in liquid form, the pharmaceutical composition contains from about 0.5 to 90% by weight of the binding agent, and preferably from about 1 to 50% of the binding agent.

[0147] When a therapeutically effective amount of an IL-21/IL-21R agonist or antagonist is administered by intravenous, cutaneous, or subcutaneous injection, the

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binding agent will be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable protein solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art.

A preferred pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection should contain, in addition to a binding agent, an isotonic vehicle such as sodium chloride injection, Ringer's injection, dextrose injection, dextrose and sodium chloride injection, lactated Ringer's injection, or other vehicles as known in the art. The pharmaceutical composition of the present invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additive known to those of skill in the art.

[0148] The amount of an IL-21/IL-21R agonist(s) or antagonist(s) in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments that the patient has undergone. Ultimately, the attending physician will decide the amount of IL-21/IL-21R agonist or antagonist with which to treat each individual patient. Initially, the attending physician will administer low doses of the IL-21/IL-21R agonist or antagonist and observe the patient's response. Larger doses of the IL-21/IL-21R agonist or antagonist may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not generally increased further. It is contemplated that the various pharmaceutical compositions used to practice the method of the present invention should contain about 0.1 µg to about 100 mg IL-21/IL-21R agonist or antagonist per kg body weight.

[0149] The duration of intravenous therapy using the pharmaceutical composition of the present invention will vary, depending on the severity of the disease being treated and the condition and potential idiosyncratic response of each individual patient. It is contemplated that the duration of each application of the IL-21/IL-21R agonist or antagonist will be in the range of 12 to 24 hrs of continuous intravenous administration. Ultimately the attending physician will decide on the appropriate

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duration of intravenous therapy using the pharmaceutical composition of the present invention.

**[0150]** The IL-21/IL-21R agonist(s) or antagonist(s) (e.g., polynucleotides, polypeptides, antibodies and small molecules) is expected to exhibit one or more of the uses or biological activities identified herein (including those associated with assays cited herein). Uses or activities described for IL-21/IL-21R agonists and antagonists may be provided, e.g., by administration or use of proteins or antibodies, by administration or use of polynucleotides encoding proteins or antibodies (such as, for example, use in gene therapies or administration with vectors suitable for introduction of DNA), or by administering the agonistic and antagonistic small molecules described herein.

Uses of IL-21/IL-21R Antagonists and Agonists to Modulate Treg Cells and Foxp3

**[0151]** As shown in the examples, the IL-21/IL-21R pathway modulates regulatory T cells (Tregs) and Foxp3, e.g., the levels and/or activity of Treg cells and Foxp3. Agonists of IL-21 and IL-21R stimulate the levels and/or activity of Treg cells and Foxp3, while antagonists of IL-21 and IL-21R suppress the levels and/or activity of Treg cells and Foxp3. These findings provide a method for modulating the levels and/or activity of Treg cells and Foxp3, which may be used in the treatment of various disorders of the immune system, as well as disorders and conditions that would benefit from the stimulation or suppression of immunity.

**[0152]** Accordingly, disclosed herein are methods for modulating the level and/or activity of a Treg cell or a population of Treg cells by contacting a Treg cell or a population of Treg cells with an IL-21/IL-21R agonist or antagonist in an amount sufficient to enhance (in the case of an IL-21/IL-21R agonist) or decrease (in the case of an IL-21/IL-21R antagonist) the level and/or activity of the Treg cell or Treg cell population. In yet another aspect, the invention features a method for modulating the level and/or activity of Foxp3 in a mammalian subject, by contacting the target immune cell or population thereof with an IL-21/IL-21R agonist or antagonist in an amount sufficient to enhance (in the case of an IL-21/IL-21R

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agonist) or decrease (in the case of an IL-21/IL-21R antagonist) the level and/or activity of Foxp3 in the immune cell or population thereof in the mammalian subject.

It is known that CD4<sup>+</sup>CD25<sup>+</sup> and CD8<sup>+</sup> T cells express Foxp3, making them desirable immune cell targets for Foxp3 regulation in a mammalian subject (Banham et al. (2006) *Eur. J. Immunol.* 36:2832-36; Ahmadzadeh et al. (2007) *J. Immunother.* 30:294-302; Yi et al. (2006) *Cell. Mol. Immuno.* 3:189-95; Pfoertner et al. (2006) *Genome Biol.* 7(7):R54).

[0153] The IL-21/IL-21R axis controls a complex range of immune processes through both positive and negative regulatory effects on lymphoid cells. Increased expression of IL-21 has been detected in several mouse models of autoimmunity, e.g., the BXSB.B6-Yaa<sup>+</sup>/J mouse, which is a model of SLE (Ozaki et al. (2004) *supra*), the nonobese diabetic (NOD) mouse, which is a model of autoimmune diabetes (King et al. (2004) *supra*), and EAMG (Vollmer et al. (2005) *supra*). Herein it is shown that blockade of IL-21 aggravates the symptoms of EAE in SJL/J mice. Furthermore, blockade of IL-21 enhanced PLP<sub>139-151</sub>-specific T cell proliferation, which is associated with an enhanced Th1/Th17 response and pronounced CNS inflammation. Therefore, the IL-21/IL-21R pathway is a major determinant for the magnitude of autoreactive T cell expansion in the periphery, and inflammation within the CNS, in SJL/J mice.

[0154] In SJL/J mice, PLP<sub>139-151</sub> is the immunodominant encephalitogenic epitope and it induces severe EAE with a protracted and relapsing-remitting course (Greer et al. (1996) *J. Immunol.* 156:371-79). The actual genes and cellular mechanisms determining susceptibility in SJL/J mice have not been fully elucidated.

[0155] Tregs are important regulators in autoimmunity. The activation and maintenance of these cells is largely influenced by IL-2 (Malek and Bayer (2004) *Nat. Rev. Immunol.* 4:665-74; Apostolou et al. (2002) *Nat. Immunol.* 3:756-63). A recent study has shown that IL-21 has structural and functional overlaps with IL-2 and IL-15 (Leonard and Spolski (2005) *supra*). The results disclosed herein show that blockade of IL-21 significantly decreases the number of Tregs, as well as their

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expression of the transcription factor Foxp3, in the EAE model. Similarly, blockade of IL-21 reduced the expression of Foxp3 in mice expressing a GFP knocked into a Foxp3 allele. These studies therefore identify a role for IL-21 in modulating Treg cells and Foxp3 levels during an autoimmune process.

[0156] It is noteworthy that treatment with IL-21R Fc on day 7 after EAE induction produced a more pronounced impact on EAE, as well as on Tregs, than treatment initiated prior to EAE induction. Similarly, treating naive (nonimmunized) SJL/J mice with IL-21R Fc at the same dosage and frequency reduced the number of Tregs, and the expression of Foxp3, to a lesser extent (data not shown). The timing of IL-21 production at least appears to contribute to those different effects. It has been shown that immunization of mice with neuroantigen and CFA can prime autoreactive CD4<sup>+</sup> T cells, subsequently releasing IL-21 (Liu et al. (2006) *supra*). In contrast, the levels of IL-21 production by CD4<sup>+</sup> T cells from naive SJL/J was below the detection level. In the absence of IL-21 production, blockade of IL-21 has minute effects on EAE and Tregs.

[0157] The physiological actions of IL-21 are broad, with known effects on B cells, CD8<sup>+</sup> T cells, NK cells, and DCs. The results disclosed herein provide the novel and unexpected findings that IL-21 also regulates Treg cells and Foxp3.

[0158] The results herein also show that a deficiency of IL-21R influences the course of MOG<sub>35-55</sub>-induced EAE significantly. IL-21R<sup>-/-</sup> mice developed severe EAE with early onset, and quickly recovered from disease. A contribution of IL-21 to the kinetics of immune regulation in EAE was associated with temporal changes in the number and localization of both Treg cells and NK cells compartments.

[0159] The effector function of T cells largely depends on cytokines. IL-21 is a cytokine that shares properties with both Th1 and Th2 responses. Some investigators have indicated that IL-21 is a proinflammatory cytokine with a capacity for upregulating the expression of genes associated with the Th1 response (Strengell et al. (2002) *supra*), and that the overexpression of IL-21 or IL-21R is associated with accumulation of mononuclear cells (Parrish-Novak et al. (2002) *J. Leukoc. Biol.*

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72:856-63; Pelletier et al. (2004) *J. Immunol.* 173:7521-30; Vollmer et al. (2005) *supra*). Other investigators have shown that IL-21 is a cytokine that can inhibit the differentiation of Th cells into IFN- $\gamma$ -producing Th1 cells (Wurster et al. (2002) *supra*), and that IL-21R augments Th2 cells (Pesce et al. (2006) *supra*) and/or Th2 responses, e.g., in allergic airway inflammation (Frolich et al. (2007) *Blood* 109:2023-31).

[0160] In EAE, as found in the present studies, a lack of IL-21R leads to a transient increase in IFN- $\gamma$ , as well as IL-4 and IL-10 expression. As EAE progresses, however, the effects of IL-21R deficiency disappear. The timing of anti IL-21R blockade might account for different effects in other diseases; for example, in rodent models of systemic autoimmunity, administration of IL-21R Fc ameliorated disease in rheumatoid arthritis (Young et al. (2007) *Arthritis Rheum.* 56:1152-63) and reduced progression of lupus-like disease (Herber et al. (2007) *J. Immunol.* 178:3822-30).

[0161] The dynamic impact of IL-21R on Th1 or Th2 cells has not been recognized previously, and its underlying mechanism can be important for a better understanding of the biology of IL-21 *in vivo*. The set of Th17 cells, a helper T cell subset, is involved in the pathogenesis of several autoimmune diseases, such as MS and EAE (Bailey et al. (2007) *Nat. Immunol.* 8:172-80; Komiyama et al. (2006) *J. Immunol.* 177:566-73). In the present studies, no significant differences in IL-17-producing Th17 cells were detected between IL-21R<sup>-/-</sup> and control mice with EAE, but IL-21R<sup>-/-</sup> T cells did show a trend toward lower levels of IL-17 expression. This is consistent with a recent report, which showed that IL-21 may initiate an alternative pathway to amplify IL-17 induction by Th17 cells, and that IL-21R<sup>-/-</sup> T cells are defective in generating such a Th17 response (Korn et al. (2007) *Nature* 448:484-87). The present results suggest that Th17 cells did not significantly impact the phenotype of IL-21R<sup>-/-</sup> mice.

[0162] Additionally, the present studies show that the early onset and severe neurological defects of IL-21R<sup>-/-</sup> mice with EAE during the priming phase is

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associated with a defect in CD4<sup>+</sup>CD25<sup>+</sup> cells and downregulated expression of Foxp3. Significantly, the production of IL-2 can compensate for impaired IL-21, as exemplified by its rescue of defective Treg cells (which contribute to the recovery from EAE in IL-21R<sup>-/-</sup> mice).

[0163] IL-21 in the presence of IL-2 is suppressive to CD4<sup>+</sup>CD25<sup>+</sup> Treg cells *in vitro*. This finding is consistent with the finding that IL-21 counteracts CD4<sup>+</sup>CD25<sup>+</sup> Treg cell-mediated suppression of human CD4<sup>+</sup> T lymphocytes without affecting the number of Foxp3<sup>+</sup> cells or the survival of Treg cells (Peluso et al. (2007) *supra*). Another report indicated that IL-21 partially reverts the immunosuppressive activity of CD4<sup>+</sup>CD25<sup>+</sup> Treg cells isolated from tumor-draining lymph nodes (Comes et al. (2006) *J. Immunol.* 176:1750-58). Both reports were based on *in vitro* studies: one in cell culture from healthy human CD4<sup>+</sup> T lymphocytes, and the other in a tumor cell line.

[0164] A reciprocal relationship between Th17 cells and Foxp3<sup>+</sup> Treg cells has recently been proposed (Bettelli et al. (2006) *Nature* 441:235-38). In this study, the development of Th17 cells and Treg cells both require the presence of transforming growth factor- $\beta$  (TGF- $\beta$ ), but the addition of IL-6 preferentially skews the response towards Th17. In another report, the roles of IL-17 and Treg cells in autoimmunity are critically dependent on the timing and nature of the disease; this is evidenced by regulatory T cells favoring IL-17 production in animal models of systemic autoimmunity, but preventing the disease when administered early in its course via suppression of the expansion of T cells (Lohr et al. (2006) *J. Exp. Med.* 203:2785-91). In the studies presented herein, a dramatic reduction of IL-17 production in IL-21R<sup>-/-</sup> EAE mice as compared to control mice was not found. Thus, the influence of Th17/IL-17 on Treg cells in EAE and the EAE phenotype was not found to be substantial here.

[0165] It has been shown that the administration of IL-21 exacerbates EAE in B6 mice (Vollmer et al. (2005) *supra*). Additionally, depletion of NK cells increases the severity of EAE (Zhang et al. (1997) *J. Exp. Med.* 186:1677-87), and a failure of

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NK cells to home to the CNS leads to fatal EAE (Huang et al. (2006) *FASEB J.* 20:896-905). The development of NK cells depends on the action of common gamma ( $\gamma_c$ ) -dependent cytokines. IL-21 plays a role in the proliferation and maturation of NK cells (Parrish-Novak et al. (2000) *supra*) and promotes their cytotoxic activity and production of IFN- $\gamma$  (Kasaian et al. (2002) *supra*). In the EAE model presented herein, an initial reduction of peripheral numbers of NK cells was found, and then an accumulation of NK cells in the CNS was seen during the EAE recovery phase. Because IL-21R<sup>-/-</sup> NK cells maintained their suppressive activity *in vivo*, redistribution of NK cells to a different anatomical location could help to explain the clinical expression of EAE in the IL-21R<sup>-/-</sup> mice.

[0166] The present studies reveal a temporal but important impact of IL-21 signaling in the development of EAE. The dynamic changes of Treg cells, as well as an organ redistribution of NK cells, may contribute to the finding of early development and severe neurological impairment associated with rapid recovery from neurological deficit.

Uses of IL-21/IL-21R Agonists to Treat, Ameliorate or Prevent Autoimmune Disorders, Inflammatory Disorders, Transplant/Graft Rejection, Lymphopenia, and Graft-versus-Host Disease

[0167] As it has been discovered that IL-21/IL-21R enhances Treg and Foxp3 levels and activity, an IL-21/IL-21R agonist can also be used to enhance the immune regulatory activity of a Treg cell and Foxp3, and, thus, can be used to treat, ameliorate, or prevent a variety of immune disorders related to enhanced immune activity, or which would benefit from immune suppression. (See, e.g., discussions regarding Tregs and/or Foxp3 and autoimmunity in Glisic-Milosavljevic et al. (2007) *PLoS ONE* 2(1):e146; DeJaco et al. (2005) *Immunology* 117:289-300; Bellinghausen et al. (2005) *Immunology* 116:103-11; Bacchetta et al. (2006) *J. Clin. Invest.* 116:1713-22).

[0168] Accordingly, disclosed herein are methods of treating, ameliorating, or preventing an autoimmune disorder, inflammatory disorder, transplant/graft



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rejection, lymphopenia, or GvHD in a mammalian subject, comprising administering to the subject an IL-21/IL-21R agonist, wherein the IL-21/IL-21R agonist is selected from the group consisting of agonistic IL-21/IL-21R polynucleotides or fragments thereof, agonistic IL-21/IL-21R polypeptides or fragments thereof, agonistic anti-IL-21/IL-21R antibodies or fragments thereof, and agonistic small molecules, in an amount sufficient to increase the level and/or activity of a Treg cell or a population of Treg cells in the mammalian subject, thereby treating, ameliorating, or preventing the autoimmune disorder, inflammatory disorder, transplant/graft rejection, lymphopenia, or GvHD in the mammalian subject.

[0169] Nonlimiting examples of the disorders that can be treated, ameliorated, or prevented using an IL-21/IL-21R agonist include, but are not limited to, transplant rejection, diabetes mellitus, arthritis (including, but not limited to, RA, juvenile RA, osteoarthritis (OA), psoriatic arthritis), multiple sclerosis (MS), encephalomyelitis, myasthenia gravis, systemic lupus erythematosus (SLE), glomerulonephritis, autoimmune thyroiditis, dermatitis (including, but not limited to, atopic dermatitis and eczematous dermatitis), psoriasis and related skin conditions (including, but not limited to, conditions associated with UV damage (including, but not limited to, photoaging), atopic dermatitis, cutaneous T cell lymphoma (including, but not limited to, mycosis fungoides), allergic and irritant contact dermatitis, lichen planus, alopecia areata, vitiligo, ocular cicatricial pemphigoid, and urticaria), Sjögren's syndrome, Crohn's disease, ulcerative colitis, aphthous ulcer, iritis, spondyloarthropathy, ankylosing spondylitis, intrinsic asthma, allergic asthma, chronic obstructive pulmonary disease (COPD), interstitial lung fibrosis, cutaneous lupus erythematosus, scleroderma, drug eruptions, autoimmune uveitis, allergic encephalomyelitis, Wegener's granulomatosis, GvHD, hepatitis, Stevens-Johnson syndrome, idiopathic sprue, Graves' disease, sarcoidosis, liver fibrosis, primary biliary cirrhosis, uveitis posterior, and allergy (including, but not limited to, atopic allergy). Exemplary disorders that can be treated using IL-21/IL-21R agonists include, but are not limited to, MS, autoimmune-lymphoproliferative syndrome

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(ALPS), juvenile idiopathic arthritis, psoriatic arthritis, autoimmune polyendocrinopathy candidiasis-ectodermal dystrophy (APECED), hepatitis C virus-associated mixed cryoglobulinemia, polymyositis, dermatomyositis, polyglandular syndrome type II, autoimmune liver disease, SLE, Kawasaki disease, myasthenia gravis, immunodysregulation polyendocrinopathy enteropathy X-linked-syndrome (IPEX), type I diabetes, psoriasis, hypothyroidism, hemolytic anemia, thrombocytopenia, spondyloarthritis, Sjögren's syndrome, rheumatoid arthritis, inflammatory bowel disease (IBD), Crohn's disease, eczema, gastritis, thyroiditis, and contact hypersensitivity.

**[0170]** Many autoimmune disorders are the result of inappropriate activation of T cells that are reactive against self and that promote the production of cytokines and autoantibodies involved in the pathology of the diseases. Increasing the level and/or activity of regulatory T cells (Treg cells) and Foxp3 may reduce or eliminate these disease symptoms. The efficacy of an IL-21/IL-21R agonist in preventing or alleviating autoimmune and inflammatory disorders can be determined using a number of well-characterized animal models of human autoimmune diseases, etc. Examples include murine experimental autoimmune encephalitis (EAE), systemic lupus erythematosus in MRL/lpr/lpr mice or NZB hybrid mice, murine autoimmune collagen arthritis, diabetes mellitus in NOD mice and BB rats, and murine experimental myasthenia gravis (see, e.g., Paul ed., *Fundamental Immunology*, Raven Press, New York (1989) pp. 840-56).

#### Uses of IL-21/IL-21R Antagonists to Treat, Ameliorate, or Prevent Cancers and Infectious Disorders

**[0171]** Because it has been discovered that IL-21/IL-21R enhances Treg and Foxp3 levels and activity, an IL-21/IL-21R antagonist can be used to suppress the immune regulatory activity of Treg and Foxp3, and, thus, can be used to treat, ameliorate, or prevent a variety of immune disorders related to suppressed immune activity, or which would benefit from immune enhancement. Accordingly, disclosed herein are methods of treating, preventing, or ameliorating a cancer or an infectious disorder in

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a mammalian subject, comprising administering to the subject an IL-21/IL-21R antagonist selected from the group consisting of antagonistic IL-21/IL-21R polynucleotides or fragments thereof, antagonistic IL-21/IL-21R polypeptides or fragments thereof, antagonistic anti-IL-21/IL-21R antibodies or fragments thereof, and antagonistic small molecules, in an amount sufficient to suppress the level and/or activity of a Treg cell or a population of Treg cells in the mammalian subject, thereby treating, ameliorating, or preventing the cancer or infectious disorder in the mammalian subject.

[0172] Nonlimiting examples of the disorders that can be treated, ameliorated, or prevented using an IL-21/IL-21R antagonist include cancers (including, but not limited to, soft tissue and solid tissue cancers and metastatic lesions) and infectious disorders (Beyer and Schultze (2006) *Blood* 108:804-11; Kish et al. (2007) *J. Leukocyte Biol.* 82:1-8; Clarke et al. (2006) *PLoS ONE* 1:e129; Beyer et al. (2005) *Blood* 106:2018-25; Knutson (2006) *Future Oncol.* 2:379-89; Holmes et al. (2007) *J. Biol. Chem.* 282:15973-80). Examples of cancers include, but are not limited to, sarcomas, melanomas, lymphomas, leukemias, neuroblastomas, colorectal cancer, breast cancer, ovarian cancer, lung cancer, B cell Hodgkin lymphoma, non-Hodgkin's lymphoma, melanoma, chronic lymphocytic leukemia, myeloid leukemia, gastrointestinal malignancies (e.g., gastric carcinoma), squamous-cell carcinoma of the head and neck, hepatocellular carcinoma, and renal cancer.

[0173] Examples of infectious disorders include, but are not limited to, infectious diseases caused by viral, bacterial, fungal, or other infection, e.g., infections by human immunodeficiency virus (HIV), simian immunodeficiency virus, respiratory syncytial virus, parainfluenza virus types 1-3, influenza virus, herpes simplex virus, human cytomegalovirus, hepatitis A virus, hepatitis B virus, hepatitis C virus, human papillomavirus, poliovirus, rotavirus, calicivirus, measles virus, mumps virus, rubella virus, adenovirus, rabies virus, canine distemper virus, rinderpest virus, coronavirus, parvovirus, infectious rhinotracheitis viruses, feline leukemia virus, feline infectious peritonitis virus, avian infectious bursal disease virus, Newcastle

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disease virus, Marek's disease virus, porcine respiratory and reproductive syndrome virus, equine arteritis virus, encephalitis viruses, *Haemophilus influenza* (both typable and nontypable), *Haemophilus somnus*, *Moraxella catarrhalis*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Enterococcus faecalis*, *Helicobacter pylori*, *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Chlamydia pneumoniae*, *Chlamydia psittaci*, *Bordetella pertussis*, *Salmonella typhi*, *Salmonella typhimurium*, *Salmonella choleraesuis*, *Escherichia coli*, *Shigella*, *Vibrio cholerae*, *Corynebacterium diphtheriae*, various mycobacteria, *Proteus mirabilis*, *Proteus vulgaris*, *Staphylococcus aureus*, *Clostridium tetani*, *Leptospira interrogans*, *Borrelia burgdorferi*, *Pasteurella haemolytica*, *Pasteurella multocida*, *Actinobacillus pleuropneumoniae*, *Mycoplasma gallisepticum*, Malaria, *Leishmania major*, *Ascaris*, *Trichuris*, *Giardia*, *Schistosoma*, *Cryptosporidium*, *Trichomonas*, *Toxoplasma gondii*, *Pneumocystis carinii*, *Aspergillus*, *Blastomyces*, *Candida*, *Coccidioides*, *Cryptococcus* and *Histoplasma*.

Uses of IL-21/IL-21R Agonists to Treat, Ameliorate, or Prevent Transplant/Graft Rejection

[0174] Transplant/graft rejection occurs when the immune system of the host organism raises an immune response against nonself antigens in a transplanted tissue, e.g., syngeneic, allogeneic, or xenogeneic tissue. Rejection can be mediated, for example, by antibodies, lymphocytes, or both, and can manifest itself in a variety of different ways, including, e.g., hyperacute rejection (e.g., during the early post-transplant period), acute rejection, and chronic rejection (generally, a slowly developing process causing a progressive decline in graft function). Rejection is often accompanied by inflammation and can result in the damage and/or failure of the transplanted tissue or organ, e.g., vasculopathy, fibrosis, or a loss of organ function. During rejection, the host may experience general discomfort, pain or swelling in the area of the transplant, and/or fever. Organ and tissue transplants can be monitored for rejection, e.g., by examination of biopsies for signs of rejection, or

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by assessing organ function. Histopathological signs of rejection include, e.g., increased expression of HLA class II antigens, e.g., in renal tubular cells following kidney transplantation. Liver function, e.g., can be assessed by measuring serum levels of bilirubin and hepatic enzymes, e.g., alkaline phosphatase; kidney function can be assessed, e.g., by measuring serum creatine levels.

[0175] As it has been discovered that IL-21/IL-21R enhances Treg and Foxp3 levels and activity, an IL-21/IL-21R agonist can also be used to enhance the immune regulatory activity of Treg and Foxp3, and, thus, can be used to treat, ameliorate, or prevent transplant/graft rejection. Downregulating or preventing immune functions, e.g., using IL-21/IL-21R agonists, will be useful in situations of tissue, skin, and organ transplantation, and in GvHD. IL-21/IL-21R agonists can be used to prevent, ameliorate, or treat tissue/graft rejection or symptoms associated with rejection, e.g., before, during, or after transplantation of an organ, tissue, or cells, e.g., heart, lung, liver, kidney, pancreas, or bone marrow.

[0176] Accordingly, disclosed herein are methods of transplanting/grafting an organ, tissue, cell, or group of cells to a mammalian subject comprising the steps of: (a) administering to the subject an IL-21/IL-21R agonist selected from the group consisting of agonistic IL-21/IL-21R polynucleotides or fragments thereof, agonistic IL-21/IL-21R polypeptides or fragments thereof, agonistic anti-IL-21/IL-21R antibodies or fragments thereof, and agonistic small molecules, in an amount sufficient to reduce the risk of transplant/graft rejection; and (b) transplanting/grafting an organ, tissue, cell or group of cells to the subject, wherein the transplanting/grafting step (b) occurs either before, during, or after the administering step (a).

[0177] Additionally disclosed herein are methods for treating, preventing or ameliorating transplant/graft rejection in a mammalian transplant/graft recipient comprising: (a) detecting a symptom of transplant/graft rejection in a transplant/graft recipient; and (b) administering to the transplant/graft recipient an IL-21/IL-21R agonist selected from the group consisting of agonistic IL-21/IL-21R

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polynucleotides or fragments thereof, agonistic IL-21/IL-21R polypeptides or fragments thereof, agonistic anti-IL-21/IL-21R antibodies or fragments thereof, and agonistic small molecules.

[0178] Inhibiting T cell function via stimulation of Tregs and Foxp3 may reduce tissue destruction during tissue transplantation. Typically, in tissue transplants, rejection of the transplant is initiated through its recognition as foreign by T cells, followed by an immune reaction that destroys the transplant. The administration of an IL-21/IL-21R agonist, alone or in combination with a molecule which inhibits or blocks interaction of other immune effectors prior to, during, or following transplantation, can serve to reduce immune responses.

[0179] The efficacy of IL-21/IL-21R agonists in preventing tissue/organ graft/transplant rejection or GvHD can be assessed using animal models that are predictive of efficacy and dosing in humans. Examples of appropriate systems which can be used include allogeneic cardiac grafts in rats and xenogeneic pancreatic islet cell grafts in mice, both of which have been used to examine the immunosuppressive effects of CTLA4 Ig fusion proteins *in vivo*, as described in Lenschow et al. (1992) *Science* 257:789-92 and Turka et al. (1992) *Proc. Natl. Acad. Sci. U.S.A.*, 89:11102-05. IL-21/IL-21R agonists can also be evaluated in other animal models, e.g., in murine models for vascularized cardiac allografts, and full-thickness skin allografts. The model can test rejection of tissues that have full MHC mismatches, and can combine IL-21 blockade with donor specific lymphocyte transfusion. In addition, murine models of GvHD (see, e.g., Paul ed., *Fundamental Immunology*, Raven Press, New York (1989) pp. 846-47) can be used to determine the effect of IL-21/IL-21R agonists *in vivo* on the development of, e.g., GvHD or SLE. The efficacy of IL-21/IL-21R agonists in preventing organ transplant rejection or GvHD can also be assessed in combination with other therapeutic agents, e.g., an immunosuppressant, such as rapamycin, cyclosporine, or CTLA4 Ig.

#### Uses of IL-21/IL-21R Antagonists to Reduce HIV Transcription

**[0180]** As it has been discovered that IL-21/IL-21R enhances Foxp3 levels and activity, and it has been shown that Foxp3 enhances HIV-1 gene expression, IL-21/IL-21R antagonists can be used to suppress HIV gene expression and transcription. Thus, IL-21/IL-21R antagonists can be used to prevent, treat, or ameliorate acquired immune deficiency syndrome (AIDS).

**[0181]** In one embodiment, a method of decreasing the transcription of HIV in a mammalian subject comprises administering to the subject an IL-21/IL-21R antagonist selected from the group consisting of antagonistic IL-21/IL-21R polynucleotides or fragments thereof, antagonistic IL-21/IL-21R polypeptides or fragments thereof, antagonistic anti-IL-21/IL-21R antibodies or fragments thereof, and antagonistic small molecules, in an amount sufficient to suppress the level and/or activity of Foxp3 in the mammalian subject, thereby decreasing the transcription of HIV in the mammalian subject.

#### Uses of IL-21R Antagonists as Vaccines and Adjuvants, and in Vaccination Methods

**[0182]** As it has been discovered that IL-21/IL-21R enhances Treg and Foxp3 levels and activity, an IL-21/IL-21R antagonist can be used to suppress the immune regulatory activity of Treg and Foxp3, and, thus, can be used to increase (enhance, boost) the ability of a vaccine composition containing an antigen to elicit a protective immune response against the antigen in a mammalian subject, i.e., IL-21/IL-21R antagonists can be used as vaccines and as vaccine adjuvants. Adjuvants are immune-modulating compounds that have the ability to enhance and/or steer the development and profile of immune responses against various antigens that are themselves poorly immunogenic. Cytokines and/or lymphokines can be used as adjuvants. The appropriate selection of adjuvants can induce appropriate humoral and cellular immune responses that would not develop in the absence of adjuvant.

**[0183]** As used herein, the phrase “vaccine adjuvant” or “vaccine therapy” is intended to mean the use of an IL-21/IL-21R antagonist in combination with an antigen (e.g., viral, parasitic, and bacterial polypeptides, proteins or peptides, or

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tumor or cancer-cell polypeptides, proteins, or peptides) or polynucleotides encoding an antigen to enhance or otherwise modulate an immune response to the antigen.

For the purpose of this definition, “combination” shall mean use in conjunction with, simultaneous with (combined or uncombined), or sequentially with an antigen.

**[0184]** The term “vaccine adjuvant composition” refers to a vaccine adjuvant that additionally includes immunologically acceptable diluents or carriers in a conventional manner to prepare injectable liquid solutions or suspensions. The vaccine adjuvant composition may additionally include agents that further enhance an immune response. For example, the vaccine adjuvant composition may additionally include 3-O-deacylated monophosphoryl lipid A (MPL™) or monophosphoryl lipid A and derivatives and analogs thereof. For example, MPL™ can be used in a range of, e.g., 1-100 µg/dose.

**[0185]** The antigens used for vaccine therapy include proteins, peptides, or polypeptides derived from immunogenic and nonimmunogenic proteins, as well as any of the following: saccharides, proteins, poly- or oligonucleotides, or other macromolecular components, or fragments thereof. As used in this section, a “peptide” comprises a series of at least six amino acids and contains at least one antigenic determinant, while a “polypeptide” is a longer molecule than a peptide, but does not constitute a full-length protein. As used in this section, a “fragment” comprises a portion, but less than all, of a saccharide, protein, poly- or oligonucleotide, or other macromolecular components.

**[0186]** As used herein, the phrase “effective adjuvanting amount” means a dose of the combination of adjuvants described herein that is suitable to elicit an increased immune response in a vertebrate host. The particular dosage will depend in part upon the age, weight, and medical condition of the host, as well as on the method of administration and the antigen. Suitable doses are readily determined by persons skilled in the art.

**[0187]** The vaccine adjuvant composition of the invention can be administered to a human or nonhuman mammal or other vertebrate by a variety of routes, including,



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but not limited to, intranasal, oral, vaginal, rectal, parenteral, intradermal, transdermal (see, e. g., International application WO 98/20734, which is hereby incorporated by reference herein), intramuscular, intraperitoneal, subcutaneous, intravenous, and intraarterial. The amount of the antigen component(s) of the antigenic composition will vary depending in part upon the identity of the antigen, as well as upon the age, weight, and medical condition of the host, as well as on the method of administration. Again, suitable doses are readily determined by persons skilled in the art.

[0188] It is preferable, although not required, that the antigen and the combination of adjuvants be administered at approximately the same time. The number of doses and the dosage regimen for the antigenic composition are also readily determined by persons skilled in the art. In some instances, the adjuvant properties of the combination of adjuvants may reduce the number of doses needed and/or the time course of the dosage regimen.

[0189] The combinations of adjuvants of this invention are suitable for use in combination with a wide variety of antigens from a wide variety of pathogenic microorganisms, including but not limited to those from viruses, bacteria, fungi, or parasitic microorganisms that infect humans and nonhuman mammals or other vertebrates, or from a cancer cell or tumor cell (e.g., sarcoma, melanoma, lymphoma, leukemia, neuroblastoma, carcinoma). The antigen may comprise peptides or polypeptides derived from proteins, as well as fragments of any of the following: saccharides, proteins, poly- or oligonucleotides, cancer or tumor cells, allergens, amyloid peptide proteins, or other macromolecular components. In some instances, more than one antigen is included in the antigenic composition.

[0190] Desirable viral vaccines containing the adjuvant combinations of this invention include those directed to the prevention and/or treatment of disease caused by, without limitation, human immunodeficiency virus, simian immunodeficiency virus, respiratory syncytial virus, parainfluenza virus types 1-3, influenza virus, herpes simplex virus, human cytomegalovirus, hepatitis A virus, hepatitis B virus,

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hepatitis C virus, human papillomavirus, poliovirus, rotavirus, caliciviruses, measles virus, mumps virus, rubella virus, adenovirus, rabies virus, canine distemper virus, rinderpest virus, coronavirus, parvovirus, infectious rhinotracheitis viruses, feline leukemia virus, feline infectious peritonitis virus, avian infectious bursal disease virus, Newcastle disease virus, Marek's disease virus, porcine respiratory and reproductive syndrome virus, equine arteritis virus, and various encephalitis viruses.

In one embodiment, the IL-21/IL-21R antagonist is administered in combination with a TNF antagonist, e.g., a TNF antagonist as described herein, to treat a hepatitis C virus infection.

[0191] Desirable bacterial vaccines containing the adjuvant combinations of this invention include those directed to the prevention, amelioration, and/or treatment of disease caused by, without limitation, *Haemophilus influenza* (both typable and nontypable), *Haemophilus somnus*, *Moraxella catarrhalis*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus faecalis*, *Helicobacter pylori*, *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Chlamydia pneumoniae*, *Chlamydia psittaci*, *Bordetella pertussis*, *Salmonella typhi*, *Salmonella typhimurium*, *Salmonella choleraesuis*, *Escherichia coli*, *Shigella*, *Vibrio cholerae*, *Corynebacterium diphtheriae*, various mycobacteria, *Proteus mirabilis*, *Proteus vulgaris*, *Staphylococcus aureus*, *Clostridium tetani*, *Leptospira interrogans*, *Borrelia burgdorferi*, *Pasteurella haemolytica*, *Pasteurella multocida*, *Actinobacillus pleuropneumoniae*, and *Mycoplasma gallisepticum*.

[0192] Desirable vaccines against fungal pathogens containing the adjuvant combinations of this invention include those directed to the prevention and/or treatment of disease caused by, without limitation, *Aspergillus*, *Blastomyces*, *Candida*, *Coccidioides*, *Cryptococcus* and *Histoplasma*.

[0193] Desirable vaccines against parasites containing the adjuvant combinations of this invention include those directed to the prevention and/or treatment of disease caused by, without limitation, *Leishmania major*, *Malaria*, *Ascaris*, *Trichuris*,

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*Giardia*, *Schistosoma*, *Cryptosporidium*, *Trichomonas*, *Toxoplasma gondii* and *Pneumocystis carinii*.

[0194] Desirable vaccines for eliciting a therapeutic or prophylactic anti-cancer effect in a vertebrate host, which contain the adjuvant combinations of this invention, include those utilizing a cancer cell or tumor cell antigen including, without limitation, prostate specific antigen (PSA), prostate-specific membrane antigen (PSMA), carcino-embryonic antigen (CEA), MUC-1, Her2, CA-125, MAGE-3, EGFR, HELP, GCC, CD66-c, prostasin, TMPRSS3, TADG 12 and TADG 15.

[0195] Desirable vaccines for moderating responses to allergens in a vertebrate host, which contain the adjuvant combinations of this invention, include those containing an allergen or fragment thereof. Examples of such allergens are described in U.S. Patent No. 5,830,877 and published International Patent Application No. WO 99/51259, which are hereby incorporated by reference herein in their entireties, and include pollen, insect venoms, animal dander, fungal spores and drugs (such as penicillin). The vaccines interfere with the production of IgE antibodies, a known cause of allergic reactions.

[0196] Desirable vaccines for preventing or treating disease characterized by amyloid deposition in a vertebrate host, which contain the adjuvant combinations of this invention, include those containing portions of amyloid peptide protein (APP). This disease is referred to variously as Alzheimer's disease, amyloidosis or amyloidogenic disease. Thus, the vaccines of this invention include the adjuvant combinations of this invention plus APP, as well as fragments of APP and antibodies to APP or fragments thereof.

[0197] In the case of HIV and SIV, the antigenic compositions comprise at least one protein, polypeptide, peptide or fragment derived from said protein. In some instances, multiple HIV or SIV proteins, polypeptides, peptides and/or fragments are included in the antigenic composition.

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[0198] The adjuvant combination formulations of this invention are also suitable for inclusion as an adjuvant in polynucleotide vaccines (also known as DNA vaccines). Such vaccines may further include facilitating agents such as bupivacaine (see U. S. Patent No. 5,593,972, which is hereby incorporated by reference herein in its entirety).

[0199] Disclosed herein are pharmaceutical compositions useful as vaccines, which comprise an antigen from a pathogenic microorganism (e.g., derived from a virus, bacteria, or parasite), and an effective adjuvanting amount of an IL-21/IL-21R antagonist, in a pharmaceutically acceptable carrier. Also disclosed herein are methods for increasing the ability of a vaccine composition containing an antigen to elicit a protective immune response against the antigen (e.g., derived from a cancer or tumor cell antigen (e.g., an antigen expressed on the surface of a cancer cell)) in a mammalian subject (e.g., a human), comprising administering to the mammalian subject, either simultaneously with or sequentially to the vaccine composition, an effective adjuvanting amount of an IL-21/IL-21R antagonist, such that the ability of the vaccine composition to elicit the protective immune response is increased. Additionally, disclosed herein are pharmaceutical compositions that comprise an antigen from a pathogenic microorganism (e.g., a viral, bacterial, or parasitic microorganism) or a cancer cell or tumor cell antigen in combination with an IL-21/IL-21R antagonist, in a pharmaceutically acceptable carrier.

#### Combination Therapy Using IL-21/IL-21R Agonists and Antagonists

[0200] In one embodiment, the IL-21/IL-21R antagonists or agonists, e.g., pharmaceutical compositions thereof, are administered in combination therapy, i.e., combined with other agents, e.g., therapeutic agents, which are useful for treating pathological conditions or disorders, such as immune and inflammatory disorders. The term "in combination" in this context means that the agents are given substantially contemporaneously, either simultaneously or sequentially. If given sequentially, at the onset of administration of the second compound, the first of the

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two compounds is preferably still detectable at effective concentrations at the site of treatment or in the subject.

[0201] For example, a combination therapy may include one or more IL-21/IL-21R agonists or antagonists, e.g., an antibody or an antigen-binding fragment thereof (e.g., a chimeric, humanized, human, or *in vitro*-generated antibody or antigen-binding fragment thereof) against IL-21 or IL-21R, an IL-21 fusion protein, a soluble IL-21R, a peptide inhibitor, or other small molecule inhibitor, coformulated with, and/or coadministered with, one or more additional therapeutic agents, including, but not limited to, one or more cytokine and growth factor inhibitors, immunosuppressants, anti-inflammatory agents, metabolic inhibitors, enzyme inhibitors, and/or cytotoxic or cytostatic agents, as described in more detail herein. Furthermore, one or more IL-21/IL-21R agonists or antagonists described herein may be used in combination with one or more of the therapeutic agents described herein. Such combination therapies may advantageously utilize lower dosages of the administered therapeutic agents, thus avoiding possible toxicities or complications associated with the various monotherapies. Moreover, the therapeutic agents disclosed herein may act on pathways that differ from the IL-21/IL-21R receptor pathway, and thus are expected to enhance and/or synergize with the effects of the IL-21/IL-21R agonists and antagonists.

[0202] Preferred therapeutic agents used in combination with an IL-21/IL-21R antagonist or agonist, as appropriate, are those agents that interact at different stages in the autoimmune and subsequent inflammatory response. In one embodiment, one or more IL-21/IL-21R agonists or antagonists described herein may be coformulated with, and/or coadministered with, one or more additional agents, such as other cytokine or growth factor antagonists (e.g., soluble receptors, peptide inhibitors, small molecules, ligand fusions); or antibodies or antigen-binding fragments thereof that bind to other targets (e.g., antibodies that bind to other cytokines or growth factors, their receptors, or other cell surface molecules); and anti-inflammatory cytokines or agonists thereof. Nonlimiting examples of the agents that can be used

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in combination with the IL-21/IL-21R agonists or antagonists described herein, include, but are not limited to, modulators of one or more interleukins (ILs) or their receptors, e.g., modulators of IL-1, IL-2, IL-6, IL-7, IL-8, IL-12, IL-13, IL-15, IL-16, IL-18, and IL-22, or their receptors; modulators of cytokines or growth factors or their receptors, such as tumor necrosis factor (TNF), LT, EMAP-II, GM-CSF, FGF and PDGF, or their receptors. IL-21/IL-21R agonists and antagonists can also be combined with modulators (e.g., inhibitors) of, e.g., antibodies to, cell surface molecules such as CD2, CD3, CD4, CD8, CD25, CD28, CD30, CD40, CD45, CD69, CD80 (B7.1), CD86 (B7.2), CD90, or their ligands, including CD154 (gp39 or CD40L), or LFA-1/ICAM-1 and VLA-4/VCAM-1 (Yusuf-Makagiansar et al. (2002) *Med. Res. Rev.* 22(2):146-67). Preferred modulators that can be used in combination with IL-21/IL-21R agonists or antagonists described herein include antagonists of IL-1, IL-6, IL-12, TNF $\alpha$ , IL-15, IL-17, IL-18, and IL-22.

[0203] Examples of modulators include IL-12 agonists or antagonists, such as chimeric, humanized, human, or *in vitro*-generated antibodies (or antigen-binding fragments thereof) that bind to IL-12 (preferably human IL-12), e.g., the antibody disclosed in WO 00/56772, Genetics Institute/BASF); IL-12 receptor modulators, e.g., antibodies to human IL-12 receptor; and soluble fragments of the IL-12 receptor, e.g., human IL-12 receptor. Examples of IL-6 modulators include antibodies (or antigen-binding fragments thereof) against IL-6 or its receptor, e.g., chimeric, humanized, human, or *in vitro*-generated antibodies to human IL-6 or its receptor, soluble fragments of the IL-6 receptor, and IL-6-binding proteins. Examples of IL-15 modulators include antibodies (or antigen-binding fragments thereof) against IL-15 or its receptor, e.g., chimeric, humanized, human, or *in vitro*-generated antibodies to human IL-15 or its receptor, soluble fragments of the IL-15 receptor, and IL-15-binding proteins. Examples of IL-18 modulators include antibodies, e.g., chimeric, humanized, human, or *in vitro*-generated antibodies (or antigen-binding fragments thereof), to human IL-18, soluble fragments of the IL-18 receptor, and IL-18 binding proteins (IL-18BP, Mallat et al. (2001) *Circ. Res.*

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89:e41-45). Examples of IL-1 modulators include interleukin-1-converting enzyme (ICE) inhibitors, such as Vx740, IL-1 antagonists, e.g., IL-1RA (anakinra, Amgen), sIL1RII (Immunex), and anti-IL-1 receptor antibodies (or antigen-binding fragments thereof).

[0204] Examples of TNF modulators include chimeric, humanized, human, or *in vitro*-generated antibodies (or antigen-binding fragments thereof) to TNF (e.g., human TNF $\alpha$ ), such as D2E7 (human TNF $\alpha$  antibody, U.S. 6,258,562; BASF), CDP-571/CDP-870/BAY-10-3356 (humanized anti-TNF $\alpha$  antibody; Celltech/Pharmacia), cA2 (chimeric anti-TNF $\alpha$  antibody; REMICADE<sup>™</sup>, Centocor); anti-TNF antibody fragments (e.g., CPD870); soluble fragments of the TNF receptors, e.g., p55 or p75 human TNF receptors or derivatives thereof, e.g., 75 kDa TNFR-IgG (75 kDa TNF receptor-IgG fusion protein, ENBREL<sup>™</sup>; Immunex; see, e.g., *Arthritis Rheumatism* (1994) 37:S295; *J. Invest. Med.* (1996) 44:235A), p55 kDa TNFR-IgG (55 kDa TNF receptor-IgG fusion protein (lenercept)); enzyme antagonists, e.g., TNF $\alpha$  converting enzyme (TACE) inhibitors (e.g., an alpha-sulfonyl hydroxamic acid derivative, WO 01/55112, and N-hydroxyformamide TACE inhibitor GW 3333, -005, or -022); and TNF-bp/s-TNFR (soluble TNF binding protein; see, e.g., *Arthritis Rheumatism* (1996) 39(9)(supplement):S284; *Amer. J. Physiol. - Heart Circ. Physiol.* (1995) 268:37-42). Preferred TNF antagonists are soluble fragments of the TNF receptors, e.g., p55 or p75 human TNF receptors or derivatives thereof, e.g., 75 kDa TNFR-IgG, and TNF- $\alpha$  converting enzyme (TACE) inhibitors.

[0205] In other embodiments, the IL-21/IL-21R agonists or antagonists described herein can be administered in combination with one or more of the following: IL-13 modulators (i.e., agonists and antagonists), e.g., soluble IL-13 receptors (sIL-13) and/or antibodies against IL-13; IL-2 modulators, e.g., DAB 486-IL-2 and/or DAB 389-IL-2 (IL-2 fusion proteins; Seragen; see, e.g., *Arthritis Rheumatism* (1993) 36:1223), and/or antibodies to IL-2R, e.g., anti-Tac (humanized anti-IL-2R; Protein Design Labs, *Cancer Res.* (1990) Mar 1; 50(5):1495-502). Yet another combination

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includes IL-21/IL-21R agonists or antagonists in combination with nondepleting anti-CD4 inhibitors (IDEC-CE9.1/SB 210396 (nondepleting primatized anti-CD4 antibody; IDEC/SmithKline)). Yet other preferred combinations include modulators (e.g., agonists or antagonists) of the costimulatory pathway CD80 (B7.1) or CD86 (B7.2) including antibodies, soluble receptors or agonist/antagonistic ligands; as well as p-selectin glycoprotein ligand (PSGL), anti-inflammatory cytokines, e.g., IL-4 (DNAX/Schering); IL-10 (SCH 52000; recombinant IL-10 DNAX/Schering); IL-13 and TGF, and antagonists and agonists thereof (e.g., agonistic antibodies).

**[0206]** In other embodiments, one or more IL-21/IL-21R agonist or antagonist can be coformulated with, and/or coadministered with, one or more anti-inflammatory drugs, immunosuppressants, or metabolic or enzymatic inhibitors. Nonlimiting examples of the drugs or inhibitors that can be used in combination with the IL-21/IL-21R agonists or antagonists described herein, include, but are not limited to, one or more of: nonsteroidal anti-inflammatory drug(s) (NSAIDs), e.g., ibuprofen, tenidap (see, e.g., *Arthritis Rheumatism* (1996) 39(9)(supplement):S280)), naproxen (see, e.g., *NeuroReport* (1996) 7:1209-13), meloxicam, piroxicam, diclofenac, and indomethacin; sulfasalazine (see, e.g., *Arthritis Rheumatism* (1996) 39(9)(supplement):S281); corticosteroids, such as prednisolone; cytokine suppressive anti-inflammatory drug(s) (CSAIDs); inhibitors of nucleotide biosynthesis, e.g., inhibitors of purine biosynthesis, folate antagonists (e.g., methotrexate (N-[4-[(2,4-diamino-6-pteridiny)methyl]methylamino]benzoyl]-L-glutamic acid); and inhibitors of pyrimidine biosynthesis, e.g., dihydroorotate dehydrogenase (DHODH) inhibitors (e.g., leflunomide (see, e.g., *Arthritis Rheumatism* (1996) 39(9)(supplement):S131; *Inflammation Research* (1996) 45:103-07)). Preferred therapeutic agents for use in combination with IL-21/IL-21R antagonists include NSAIDs, CSAIDs, DHODH inhibitors (e.g., leflunomide), and folate antagonists (e.g., methotrexate).

**[0207]** Examples of additional modulators include one or more of: corticosteroids (oral, inhaled and local injection); immunosuppressants, e.g., cyclosporin, tacrolimus



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(FK-506); and mTOR modulators, e.g., sirolimus (rapamycin) or rapamycin derivatives, e.g., soluble rapamycin derivatives (e.g., ester rapamycin derivatives, e.g., CCI-779 (Elit (2002) *Curr. Opin. Investig. Drugs* 3(8):1249-53; Huang et al. (2002) *Curr. Opin. Investig. Drugs* 3(2):295-304); agents that interfere with signaling by proinflammatory cytokines such as TNF $\alpha$  or IL-1 (e.g., IRAK, NIK, IKK, p38 or MAP kinase inhibitors); Cox-2 inhibitors, e.g., celecoxib and variants thereof, MK-966 (see, e.g., *Arthritis Rheumatism* (1996) 39(9)(supplement):S81); phosphodiesterase inhibitors, e.g., R973401 (phosphodiesterase Type IV inhibitor; see, e.g., *Arthritis Rheumatism* (1996) 39(9)(supplement):S282); phospholipase modulators, e.g., inhibitors of cytosolic phospholipase 2 (cPLA2) (e.g., trifluoromethyl ketone analogs (U.S. 6,350,892)); modulators of vascular endothelial cell growth factor or growth factor receptor, e.g., VEGF inhibitor and/or VEGF-R inhibitor; and modulators of angiogenesis. Preferred therapeutic agents for use in combination with IL-21/IL-21R agonists or antagonists include immunosuppressants, e.g., cyclosporin, tacrolimus (FK-506); and mTOR inhibitors, e.g., sirolimus (rapamycin) or rapamycin derivatives, e.g., soluble rapamycin derivatives (e.g., ester rapamycin derivatives, e.g., CCI-779; Cox-2 inhibitors, e.g., celecoxib and variants thereof; and phospholipase inhibitors, e.g., inhibitors of cytosolic phospholipase 2 (cPLA2) (e.g., trifluoromethyl ketone analogs)).

**[0208]** Additional examples of therapeutic agents that can be combined with an IL-21/IL-21R agonist or antagonist include one or more of: 6-mercaptopurines (6-MP); azathioprine sulphasalazine; mesalazine; olsalazine chloroquine/hydroxychloroquine; pencillamine; aurothiornalate (intramuscular and oral); azathioprine; colchicine; beta-2 adrenoreceptor agonists (salbutamol, terbutaline, salmeterol); xanthines (theophylline, aminophylline); cromoglycate; nedocromil; ketotifen; ipratropium and oxitropium; mycophenolate mofetil; adenosine agonists; antithrombotic agents; complement inhibitors; and adrenergic agents.

**[0209]** The IL-21/IL-21R agonists or antagonists disclosed herein in combination with other therapeutic agents may be used to treat, ameliorate or prevent the

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disorders discussed herein, as well as additional related disorders that are well known to those of skill in the art.

[0210] Nonlimiting examples of agents for treating, ameliorating, or preventing arthritic disorders (e.g., RA, inflammatory arthritis, juvenile RA, OA, and psoriatic arthritis), with which an IL-21/IL-21R agonist can be combined, include one or more of the following: IL-12 modulators (e.g., antagonists) as described herein, NSAIDs; CSAIDs; TNFs, e.g., TNF $\alpha$ , modulators (e.g., antagonists) as described herein; nondepleting anti-CD4 antibodies as described herein; IL-2 modulators (e.g., antagonists) as described herein; anti-inflammatory cytokines, e.g., IL-4, IL-10, IL-13, and TGF $\alpha$ , or agonists thereof; IL-1 or IL-1 receptor modulators as described herein; phosphodiesterase modulators as described herein; Cox-2 modulators (e.g., inhibitors) as described herein; iloprost (see, e.g., *Arthritis Rheumatism* (1996) 39(9)(supplement):S82); methotrexate; thalidomide (see, e.g., *Arthritis Rheumatism* (1996) 39(9)(supplement):S282) and thalidomide-related drugs (e.g., Celgen); leflunomide; modulators (e.g., inhibitors) of plasminogen activation, e.g., tranexamic acid (see, e.g., *Arthritis Rheumatism* (1996) 39(9)(supplement):S284); cytokine modulators, e.g., T-614 (see, e.g., *Arthritis Rheumatism* (1996) 39(9)(supplement):S282); prostaglandin E1 (see, e.g., *Arthritis Rheumatism* (1996) 39(9)(supplement):S282); azathioprine (see, e.g., *Arthritis Rheumatism* (1996) 39(9)(supplement):S281); a modulator (e.g., an inhibitor) of interleukin-1 converting enzyme (ICE); zap-70 and/or lck modulators (e.g., an inhibitor of zap-70 or lck); a modulator (e.g., an inhibitor) of vascular endothelial cell growth factor or vascular endothelial cell growth factor receptor as described herein; an inhibitor of angiogenesis as described herein; corticosteroid anti-inflammatory drugs (e.g., SB203580); TNF-converterase inhibitors; IL-11 (see, e.g., *Arthritis Rheumatism* (1996) 39(9)(supplement):S296); IL-13 (see, e.g., *Arthritis Rheumatism* (1996) 39(9)(supplement):S308); IL-17 modulators (see, e.g., *Arthritis Rheumatism* (1996) 39(9)(supplement):S120); gold; penicillamine; chloroquine; hydroxychloroquine; chlorambucil; cyclophosphamide; cyclosporine; total lymphoid irradiation; anti-

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thymocyte globulin; CD5-toxins; orally administered peptides and collagen; lobenzarit disodium; cytokine-regulating agents (CRAs) HP228 and HP466 (Houghten Pharmaceuticals, Inc.); ICAM-1 antisense phosphorothioate oligodeoxynucleotides (ISIS 2302; Isis Pharmaceuticals, Inc.); soluble complement receptor 1 (TP10; T Cell Sciences, Inc.); prednisone; orgotein; glycosaminoglycan polysulphate; minocycline; anti-IL-2R antibodies; marine and botanical lipids (fish and plant seed fatty acids; see, e.g., DeLuca et al. (1995) *Rheum. Dis. Clin. North Am.* 21:759-77); auranofin; phenylbutazone; meclofenamic acid; flufenamic acid; intravenous immune globulin; zileuton; mycophenolic acid (RS-61443); tacrolimus (FK-506); sirolimus (rapamycin); amiprilose (therafectin); cladribine (2-chlorodeoxyadenosine); and azaribine. Preferred combinations include one or more IL-21/IL-21R modulators in combination with methotrexate or leflunomide, and in moderate or severe rheumatoid arthritis cases, cyclosporine.

[0211] Exemplary modulators to use in combination with IL-21/IL-21R agonists to treat, ameliorate, or prevent arthritic disorders include TNF modulators (e.g., antagonists (e.g., chimeric, humanized, human, or *in vitro*-generated antibodies, or antigen-binding fragments thereof, that bind to TNF; soluble fragments of a TNF receptor, e.g., p55 or p75 human TNF receptor or derivatives thereof, e.g., 75 kDa TNFR-IgG (75 kDa TNF receptor-IgG fusion protein, ENBREL™), p55 kDa TNF receptor-IgG fusion protein; TNF enzyme antagonists, e.g., TNF $\alpha$  converting enzyme (TACE) inhibitors); antagonists of IL-6, IL-12, IL-15, IL-17, IL-18, IL-22; T cell and B cell depleting agents (e.g., anti-CD4 or anti-CD22 antibodies); small molecule inhibitors, e.g., methotrexate and leflunomide; sirolimus (rapamycin) and analogs thereof, e.g., CCI-779; Cox-2 and cPLA2 inhibitors; NSAIDs; p38 inhibitors, TPL-2, Mk-2 and NF $\kappa$ B inhibitors; RAGE or soluble RAGE; P-selectin or PSGL-1 inhibitors (e.g., small molecule inhibitors, antibodies thereto, e.g., antibodies to P-selectin); estrogen receptor beta (ERB) agonists, FKB-506/FK-506, remicade, rituxan, HUMIRA™, gold, sulphasalazine, hydroxychloroquinone, minocycline, anakinra, CTLA4Ig, antibodies to

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costimulatory molecules (e.g., antibodies to B7.1, B7.2, ICOSL, ICOS, CD28, and CTLA4), and ERB-NF $\kappa$ B antagonists. Most preferred additional therapeutic agents that can be coadministered and/or coformulated with one or more IL-21/IL-21R agonists include one or more of: a soluble fragment of a TNF receptor, e.g., p55 or p75 human TNF receptor or derivatives thereof, e.g., 75 kDa TNFR-IgG (75 kDa TNF receptor-IgG fusion protein, ENBREL<sup>TM</sup>); methotrexate, leflunomide, or a sirolimus (rapamycin) or an analog thereof, e.g., CCI-779.

[0212] Nonlimiting examples of agents for treating, ameliorating, or preventing multiple sclerosis (MS) with which an IL-21/IL-21R agonist can be combined include the following: interferons, e.g., interferon-alpha 1a (e.g., AVONEX<sup>TM</sup>; Biogen) and interferon-1b (BETASERON<sup>TM</sup>; Chiron/Berlex); Copolymer 1 (Cop-1; COPAXONE<sup>TM</sup>; Teva Pharmaceutical Industries, Inc.); hyperbaric oxygen; intravenous immunoglobulin; cladribine; TNF antagonists as described herein; corticosteroids; prednisolone; methylprednisolone; azathioprine; cyclophosphamide; cyclosporine; methotrexate; 4-aminopyridine; and tizanidine. Additional antagonists that can be used in combination with IL-21/IL-21R modulators include antibodies to or antagonists of other human cytokines or growth factors, for example, TNF, LT, IL-1, IL-2, IL-6, IL-7, IL-8, IL-12 IL-15, IL-16, IL-18, EMAP-11, GM-CSF, FGF, and PDGF. IL-21/IL-21R modulators as described herein can be combined with antibodies to cell surface molecules such as CD2, CD3, CD4, CD8, CD25, CD28, CD30, CD40, CD45, CD69, CD80, CD86, CD90 or their ligands. The IL-21/IL-21R modulators may also be combined with agents, such as methotrexate, cyclosporine, FK506, rapamycin, mycophenolate mofetil, leflunomide, NSAIDs, corticosteroids such as prednisolone, phosphodiesterase inhibitors, adenosine agonists, antithrombotic agents, complement inhibitors, adrenergic agents, agents that interfere with signaling by proinflammatory cytokines as described herein, IL-1b converting enzyme inhibitors (e.g., Vx740), anti-P7s, PSGL, TACE inhibitors, T cell signaling inhibitors such as kinase inhibitors, metalloproteinase inhibitors, sulfasalazine, azathioprine, 6-mercaptopurines, angiotensin converting enzyme

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inhibitors, soluble cytokine receptors and derivatives thereof, as described herein, and anti-inflammatory cytokines (e.g. IL-4, IL-10, IL-13 and TGF).

[0213] Preferred examples of therapeutic agents for MS with which the IL-21/IL-21R agonists can be combined include interferon-b, for example, IFN $\beta$ -1a and IFN $\beta$ -1b; copaxone, corticosteroids, IL-1 inhibitors, TNF inhibitors, antibodies to CD40 ligand and CD80, IL-12 antagonists, natalizumab (Tysabri), FTY720, and SIP1 inhibitors.

[0214] Nonlimiting examples of agents for treating, ameliorating, or preventing inflammatory bowel disease (e.g., Crohn's disease; ulcerative colitis) with which an IL-21/IL-21R agonist can be combined include the following: budesonide; epidermal growth factor; corticosteroids; cyclosporin, sulfasalazine; aminosalicylates; 6-mercaptopurine; azathioprine; metronidazole; lipoxxygenase inhibitors; mesalamine; olsalazine; balsalazide; antioxidants; thromboxane inhibitors; IL-1 receptor antagonists; anti-IL-1 monoclonal antibodies; anti-IL-6 monoclonal antibodies; growth factors; elastase inhibitors; pyridinyl-imidazole compounds; anti-IL-13; TNF antagonists as described herein; IL-4, IL-10, IL-13, and/or TGF $\beta$  cytokines or agonists thereof (e.g., agonist antibodies); IL-11; glucuronide- or dextran-conjugated prodrugs of prednisolone, dexamethasone or budesonide; ICAM-1 antisense phosphorothioate oligodeoxynucleotides (ISIS 2302; Isis Pharmaceuticals, Inc.); soluble complement receptor 1 (TP10; T Cell Sciences, Inc.); slow-release mesalazine; methotrexate; antagonists of platelet activating factor (PAF); ciprofloxacin; and lignocaine.

[0215] In one embodiment, an IL-21/IL-21R modulator (i.e., agonist or antagonist) can be used in combination with one or more antibodies directed at other targets involved in regulating immune responses, e.g., transplant rejection, GvHD, or other immune response-related disorders. Nonlimiting examples of agents for treating or preventing immune responses with which an IL-21/IL-21R modulator can be combined include the following: antibodies against cell surface molecules or their ligands, including but not limited to CD25 (IL-2 receptor-a), CD11a (LFA-1), CD54

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(ICAM-1), CD4, CD40, CD40L, CD45, CD28/CTLA4, CD80 (B7.1) and/or CD86 (B7.2). In yet another embodiment, an IL-21/IL-21R modulator can be used in combination with corticosteroids; sirolimus (rapamycin) and analogs thereof, e.g., CCI-779; cyclosporin A; FK506; FTY720; azathioprine; cyclophosphamide; methotrexate; anti-IL-2R antibodies, e.g., basiliximab, daclizumab; cA2 (chimeric anti-TNF $\alpha$  antibody; REMICADE<sup>™</sup>, Centocor); anti-CD3 antibodies (e.g., muromonab-CD3); Copolymer 1 (Cop-1; COPAXONE<sup>™</sup>; Teva Pharmaceutical Industries, Inc.); deoxyspergualin; and mycophenolate mofetil.

[0216] Nonlimiting examples of agents for treating, ameliorating, or preventing psoriasis and other skin conditions with which an IL-21/IL-21R modulator can be combined include one or more of the following: inhibitors of CD2 or LFA-3 interactions (e.g., soluble CD2- or LFA-polypeptides, such as Fc fusions, or antibodies against CD2 or LFA-3), cyclosporin A, anti-TNF, prednisone, FK506, anti-IL-12, anti-p40, anti-IL-23, methotrexate, PUVA, UV light, steroids, retinoids, interferon, or nitrogen mustard. Examples of preferred agents that can be used in combination with an IL-21/IL-21R agonist include cyclosporine A and methotrexate.

[0217] Nonlimiting examples of agents for treating, ameliorating, or preventing asthma with which an IL-21/IL-21R modulator can be combined include one or more of the following: inhaled bronchodilators, e.g., pirbuterol, bitolterol, metaproterenol; beta 2-adrenoceptor agonists, e.g., albuterol, terbutaline, salmeterol, formoterol; antimuscarinics, e.g., ipratropium, anti-IL-13, oxitropium; systemic corticosteroids, e.g., prednisone, prednisolone, dexamethasone; inhaled corticosteroids, e.g., fluticasone, budesonide, beclomethasone, mometasone; leukotriene antagonists, e.g., montelukast sodium, zafirlukast; mast cell stabilizers, e.g., cromolyn sodium, nedocromil; omalizumab (XOLAIR<sup>™</sup>; Genentech/Novartis); or Cox-2 inhibitors, as described herein.

[0218] Nonlimiting examples of agents for treating, ameliorating, or preventing lupus (e.g., SLE) with which an IL-21/IL-21R agonist can be combined include one or more of the following: IL-6/IL-6R antagonists, e.g. anti-IL-6 or anti-IL-6R

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antibodies; NSAIDs; corticosteroids, e.g., dexamethasone, hydrocortisone, methylprednisolone, prednisolone, prednisone; azathioprine, cyclophosphamide, hydroxychloroquine, Rituxan, or chloroquine.

[0219] Nonlimiting examples of agents for treating, ameliorating, or preventing cancers with which an IL-21/IL-21R antagonist can be combined include, without limitation, CCI-779, rapamycin, rapamycin analogs, cyclophosphamide (CYTOXAN®), methotrexate (RHEUMATREX®), 5-fluorouracil (5-FU), Doxorubicin (ADRIAMYCIN®), bleomycin (BLENOXANE®), taxol (PACLITAXEL®), oncovin (VINCRIStINE®), cytarabine (cytosine arabinoside, ARA-C®, CYTOSAR®), mitoxantrone (NOVANTRONE®), and chlorodeoxyadenosine (CLADRIBINE®).

[0220] Nonlimiting examples of agents for treating, ameliorating, or preventing infectious disorders with which an IL-21/IL-21R antagonist can be combined include, without limitation, antibiotics, anti-viral compounds, penicillins (such as penicillin and amoxicillin), cephalosporins (such as cephalexin (KEFLEX®), macrolides (such as erythromycin (E-MYCIN®), clarithromycin (BIAXIN®), and azithromycin (ZITHROMAX®)), fluoroquinolones (such as ciprofloxacin (CIPRO®), levofloxacin (LEVAQUIN®), and ofloxacin (FLOXIN®)), sulfonamides (such as co-trimoxazole (BACTRIM®) and trimethoprim (PROLOPRIM®)), tetracyclines (such as tetracycline (SUMYCIN®, PANMYCIN®) and doxycycline (VIBRAMYCIN®)), amino glycosides (such as gentamicin (GARAMYCIN®) and tobramycin (TOBREX®)), acyclovir (ZOVIRAX®), famcyclovir (FAMVIR®), valacyclovir (VALTREX®), amantadine (SYMMETREL®), rimantadine (FLUMADINE®), ribavirin (REBETOL® or VIRAZOLE®), AZT (azidothymidine, RETROVIR®, ZIDOVUDINE®), and COMBIVIR® (a combination of the medications zidovudine and lamivudine).

[0221] Another aspect of the present invention accordingly relates to kits for carrying out the combined administration of the IL-21/IL-21R agonists and antagonists with other therapeutic compounds. In one embodiment, the kit

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comprises one or more binding agents formulated in a pharmaceutical carrier, and at least one agent, e.g., therapeutic agent, formulated as appropriate, in one or more separate pharmaceutical preparations.

#### Assays for Measuring the Activity of IL-21/IL-21R Agonists and Antagonists

[0222] The activity of a candidate IL-21/IL-21R agonist or antagonist for use in the disclosed methods may be assayed based on the ability of the candidate compound to modulate cytokine production and cell proliferation/differentiation using any one of a number of routine factor-dependent cell proliferation assays for cell lines including, without limitation, 32D, DA2, DA1G, T10, B9, B9/11, BaF3, MC9/G, M+(preB M+), 2E8, RB5, DA1, 123, T1165, HT2, CTLL2, TF-1, Mo7e and CMK.

[0223] Assays for T cell or thymocyte proliferation include, without limitation, those described in: *Current Protocols in Immunology*, Eds. Coligan, Kruisbeek, Margulies, Shevach, and Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, *In vitro* assays for Mouse Lymphocyte Function; 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al. (1986) *J. Immunol.* 137:3494-500; Bertagnolli et al. (1990) *J. Immunol.* 145:1706-12; Bertagnolli et al. (1991) *Cell. Immunol.* 133:327-41; Bertagnolli et al. (1992) *J. Immunol.* 149:3778-83; Bowman et al. (1994) *J. Immunol.* 152:1756-61. Assays for cytokine production and/or proliferation of spleen cells, lymph node cells or thymocytes include, without limitation, those described in: Polyclonal T cell stimulation, Kruisbeek and Shevach, In *Current Protocols in Immunology*, Coligan et al., eds., Vol. 1, pp. 3.12.1-3.12.14, John Wiley and Sons, Toronto, 1994; and Measurement of mouse and human Interferon gamma, Schreiber, In *Current Protocols in Immunology* Coligan et al., eds. Vol. 1 pp. 6.8.1-6.8.8, John Wiley and Sons, Toronto, 1994.

[0224] Assays for proliferation and differentiation of hematopoietic and lymphopoietic cells include, without limitation, those described in: Measurement of Human and Murine Interleukin 2 and Interleukin 4, Bottomly, Davis, and Lipsky, in *Current Protocols in Immunology* Coligan et al., eds., Vol. 1, pp. 6.3.1-6.3.12, John



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Wiley and Sons, Toronto, 1991; deVries et al. (1991) *J. Exp. Med.* 173:1205-11; Moreau et al. (1988) *Nature* 336:690-92; Greenberger et al. (1983) *Proc. Natl. Acad. Sci. U.S.A.* 80:2931-38; Measurement of mouse and human interleukin 6, Nordan, In *Current Protocols in Immunology* Coligan et al., eds. Vol. 1 pp. 6.6.1-6.6.5, John Wiley and Sons, Toronto, 1991; Smith et al. (1986) *Proc. Natl. Acad. Sci. U.S.A.* 83:1857-61; Measurement of human Interleukin 11, Bennett, Giannotti, Clark, and Turner, In *Current Protocols in Immunology* Coligan et al., eds. Vol. 1 pp. 6.15.1 John Wiley and Sons, Toronto, 1991; Measurement of mouse and human Interleukin 9, Ciarletta, Giannotti, Clark, and Turner, In *Current Protocols in Immunology* Coligan et al., eds. Vol. 1 pp. 6.13.1, John Wiley and Sons, Toronto, 1991.

[0225] Assays for T cell-clone responses to antigens (which will identify, among others, proteins that affect APC-T cell interactions as well as direct T cell effects by measuring proliferation and cytokine production) include, without limitation, those described in: *Current Protocols in Immunology*, Ed. by Coligan et al., Greene Publishing Associates and Wiley-Interscience (Chapter 3, *In vitro* Assays for Mouse Lymphocyte Function; Chapter 6, Cytokines and Their Cellular Receptors; Chapter 7, Immunologic Studies in Humans); Weinberger et al. (1980) *Proc. Natl. Acad. Sci. U.S.A.* 77:6091-95; Weinberger et al. (1981) *Eur. J. Immun.* 11:405-11; Takai et al. (1986) *J. Immunol.* 137:3494-500; Takai et al. (1988) *J. Immunol.* 140:508-12; the contents of all of which are hereby incorporated by reference herein.

[0226] Additional assays include cellular cytotoxicity assays (see, e.g., Liu et al. (2002) *Nat. Med.* 8:185-89; McCoy et al. (1973) *Natl. Cancer Inst. Monogr.* 37:59-67).

[0227] Further assays for testing a candidate compound for its ability to function as an IL-21/IL-21R agonist or antagonist may be found in the Examples included herein, and other assays that are well known in the art.

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[0228] The entire contents of all references, patent applications, and patents cited throughout this application are hereby incorporated by reference herein. The invention will be further illustrated in the following nonlimiting examples.

### EXAMPLES

#### Example 1: Comparison of Human IL-21R, Murine IL-21, and Human IL-2R Beta Chain Polypeptide Sequences

[0229] The GAP algorithm was used to compare human (SEQ ID NO:2) and murine (SEQ ID NO:4) IL-21R polypeptides. A comparison of the murine and human IL-21R predicted protein sequences is shown in **FIG. 1**. The amino acids are 65.267% identical using the GAP algorithm. The alignment was generated by BLOSUM62 amino acid substitution matrix (Henikoff and Henikoff (1992) *Proc. Natl. Acad. Sci. U.S.A.* 89: 10915-19). Gap parameters = Gap Weight:8; Average Match = 2.912; Length Weight = 2; Average Mismatch = -2.003. The carboxy terminal tyrosine, as part of a YXXQ motif, is a potential STAT docking site.

[0230] **FIG. 2** depicts a multiple sequence alignment of the amino acids of human IL-21R (corresponding to SEQ ID NO:2), murine IL-21R (corresponding to SEQ ID NO:4), and human IL-2R beta chain (corresponding to SEQ ID NO:5) (GENBANK® Accession No. AAA59143). Full length IL-21R (about 2600bp) is 28% identical to IL-2Rβ, with higher homology (40%) in the intracellular region. Murine IL-21R is 65% identical to human IL-21R. Leader and transmembrane domains are underlined. Conserved cytokine receptor module motifs (PXPP and WSXWS (SEQ ID NO:15) motifs) are indicated by boldface type. Potential signaling regions are indicated by underlining and boldface type.

#### Example 2: Comparison of Human, Rat and Murine IL-21 Polypeptide Sequences

[0231] **FIG. 3** depicts a multiple-sequence alignment of the amino acids of human IL-21 ("Translation of hIL-21") (corresponding to SEQ ID NO:9), murine IL-21 ("mIL-21") (corresponding to SEQ ID NO:11), and rat IL-21 ("Translation of rat IL-21 CDS") (corresponding to SEQ ID NO:13). This alignment was generated by

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the program Align X (Invitrogen Life Technologies, Carlsbad, CA) using the amino acid substitution matrix BLOSUM62 with the following parameters: Gap opening penalty:10; Gap extension penalty:0.05; Gap separation penalty range:8. The underlined amino acid abbreviations represent residues conserved across all three species of human, mouse and rat. Conservation in two out of three species is indicated by boldface and underlined type, and weakly similar residues are indicated by boldface type. Overall, this alignment demonstrates that there is a 59% identity between amino acids 8 to 153 of SEQ ID NO:9; amino acids 1 to 150 of SEQ ID NO:11; and amino acids 1 to 150 of SEQ ID NO:13.

#### Example 3: IL-21 and IL-21R Expression in EAE

##### Example 3.1: Materials and Methods

[0232] SJL/J (H-2<sup>S</sup>) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). All mice were housed in animal facilities of the Barrow Neurological Institute. Only female mice (6-8 weeks old) were used. Experiments were conducted in accordance with institutional guidelines.

[0233] The murine PLP<sub>139-151</sub> peptide (HSLGKWLGHDPKF) (SEQ ID NO:16) was from Bio-Synthesis Inc. (Lewisville, TX). Murine IL-21R Fc was produced at Wyeth Research, Cambridge, MA (Pesce et al. (2006) *supra*).

[0234] EAE was induced in SJL/J (H-2<sup>S</sup>) mice as described in Tuohy et al. (1989) *J. Immunol.* 142:1523-27. Briefly, SJL/J (H-2<sup>S</sup>) mice were injected subcutaneously in the hind flank with 100 µg of PLP<sub>139-151</sub> peptide in complete Freund's adjuvant (CFA) (Difco, Detroit, MI) containing 500 µg of mycobacterium tuberculosis, supplemented by tail-base intravenous injections of 200 ng pertussis toxin (List Biologic, Campbell, CA) on day 0 and day 2. Control mice were injected with PBS. The mice were observed daily for clinical signs of disease and scored on an arbitrary scale of 0 to 5, with gradations of 0.5 for intermediate scores (Sean et al. (1998) *J. Exp. Med.* 187:1517-28): 0, no clinical signs; 1, flaccid tail; 2, hind limb weakness or abnormal gait; 3, complete hind limb paralysis; 4, complete hind limb paralysis with forelimb weakness or paralysis; 5, moribund or deceased.

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[0235] Production of IL-21 by autoreactive T cells was detected by enzyme-linked immunosorbent assay (ELISA) (DuoSet ELISA kit, R&D Systems, Minneapolis, MN). Briefly, mice were sacrificed on days 1, 3, 7, 15, 35, and 75 after EAE induction. Splenocytes were isolated from control (PBS) and EAE mice at indicated time points and mononuclear cells ( $4 \times 10^5$  cells/well) were cultured with PLP<sub>139-151</sub> peptide (10 µg/ml) for 72 hrs. Supernatants were harvested and analyzed for the presence of IL-21 by ELISA.

#### Example 3.2: Results

[0236] To assess the levels of IL-21 production by autoreactive T cells from EAE mice, IL-21 levels were measured by ELISA at days 1, 3, 7, 15, 35, and 75 after immunization with PLP<sub>139-151</sub> peptide (**FIG. 4**). From day 3 onwards, there were measurable levels of expression of IL-21 (day 3,  $19.7 \pm 1.63$  pg/ml; day 7,  $63.0 \pm 5.54$ ; day 15,  $82.8 \pm 10.28$ ; day 35,  $85.8 \pm 3.69$ ; day 75,  $83.7 \pm 6.26$ ). Data are expressed as mean  $\pm$  SEM. Levels of IL-21R expression on Tregs were also measured. Tregs expressed moderate levels of IL-21R ( $5.30 \pm 0.21$  pg/ml in EAE mice vs.  $5.20 \pm 0.07$  in PBS-treated controls), which remained relatively unchanged during the course of EAE (data not shown).

#### Example 4: *In vivo* Blockade of IL-21 Accelerates EAE

##### Example 4.1: Materials and Methods

[0237] To block IL-21 *in vivo*, 250 µg of IL-21R Fc (produced at Wyeth Research, Cambridge, MA) was injected intraperitoneally either 2 days before (day -2) or 7 days after (day +7) immunization with PLP<sub>139-151</sub>. Every 7 days thereafter, 50 µg of IL-21R Fc was injected intraperitoneally until termination of the experiment. Control mice were injected with IgG2a Ab. The seven-days post-immunization group was included in order to observe the effect of blocking IL-21 after vigorous PLP-reactive cells had been induced, but before clinical signs of EAE were apparent.

[0238] For preparation of tissue and histological staining, mice were anesthetized with pentobarbital and perfused by intracardiac puncture with 50 ml of cold PBS.

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Spinal cords were removed, frozen immediately, and stored at -80°C. Cryosections of tissues were fixed with acetone and stained with hematoxylin and eosin for visualization of inflammatory infiltrates. Histological findings were graded into four categories: 1, subpial infiltration; 2, mild perivascular cuffing; 3, extensive perivascular cuffing; 4, extensive perivascular cuffing and severe parenchymal cell infiltration (Jee and Matsumoto (2001) *Eur. J. Immunol.* 31:1800-12).

[0239] Data were evaluated by the Student's *t* test. Disease incidence and severity were analyzed by the Fisher's exact test and the Mann-Whitney's *U*-test, respectively. Histological data were analyzed using ANOVA. The *p* value was indicated if differences between two groups were statistically significant ( $<0.05$ ).

#### Example 4.2: Results

[0240] EAE induced in SJL/J (H-2<sup>S</sup>) mice is an animal model of relapsing-remitting human MS. To assess whether the IL-21/IL-21R pathway can affect development of EAE in SJL/J (H-2<sup>S</sup>) mice, IL-21 was blocked *in vivo* by using soluble IL-21R Fc in EAE mice.

[0241] The extent of EAE in the mice receiving IL-21R Fc was more severe than that of the control mice (FIG. 5A and 5B). The maximum disease clinical scores of mice receiving IL-21R Fc before and after immunization were  $2.75 \pm 0.63$  and  $3.25 \pm 0.75$ , respectively ( $p < 0.05$  and  $p < 0.001$  vs. controls ( $2.50 \pm 1.00$ ), respectively) (Table 2, below). The enhancement of EAE was more pronounced in mice receiving IL-21R Fc after immunization. Additionally, IL-21 blockade led to a relatively earlier onset of the disease ( $11.60 \pm 0.93$  days (day -2) and  $10.00 \pm 1.00$  days (day +7) after immunization). The median day of disease onset in control mice was  $14.5 \pm 3.58$  days after immunization. In agreement with the clinical data, histological findings in EAE mice receiving IL-21R Fc showed more severe CNS inflammation (FIG. 5C, middle and lower panels) compared to controls (FIG. 5C, upper panel). The mean histological scores in IL-21R Fc-treated mice were  $3.17 \pm 0.44$  (day -2) and  $3.34 \pm 0.44$  (day +7), whereas control mice had a score of  $2.50 \pm 0.02$  ( $p < 0.05$ ).

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(Table 2). Thus, the IL-21/IL-21R axis significantly affects clinical severity and CNS inflammation in EAE.

**Table 2:** *Clinical and Histological Evaluation of EAE Induced by PLP<sub>139-151</sub> in SJL/J Mice Injected with IL-21R Fc Before and After Immunization<sup>a</sup>*

Treatment	Clinical status			
	Incidence of EAE (%)	Day of onset	Maximum clinical score	Mean histological score
Control (IgG2a Ab)	95 (19/20)	14.50 ± 3.58	2.50 ± 1.00	2.50 ± 0.20
IL-21R Fc, Day -2	100 (6/6)	11.60 ± 0.93	2.75 ± 0.63*	3.17 ± 0.44*
IL-21R Fc, Day +7	100 (5/5)	10.00 ± 1.00	3.25 ± 0.75**	3.34 ± 0.44*

<sup>a</sup> Mice were induced to develop EAE and injected with either IgG2a Ab (control group) or IL-21R Fc as described above. The mice were observed for 75 days after immunization with PLP. The incidence, day of onset, and maximal disease grade were determined during the observation period. Hematoxylin and eosin-stained sections were prepared from three segments of lumbar spinal cords. Inflammatory lesion of these spinal cords was graded into four categories, as described above. Means of maximal scores were calculated for mice that showed evidence of disease. Data represent mean ± SD of total mice in each group. Statistical evaluation was performed to compare experimental groups and corresponding control groups, respectively.

\* indicates  $p < 0.05$  vs. control, \*\* indicates  $p < 0.001$  vs. control. The experiment was repeated three times with similar results.

#### Example 5: *In vivo* Blockade of IL-21 Enhances T cell Proliferation

##### Example 5.1: Materials and Methods

[0242] At the peak stage of EAE onset (day 12), mice were injected with 1.0 mg of bromodeoxyuridine (BrdU) (BD Biosciences, San Jose, CA) solution in sterile Dulbecco's phosphate-buffered saline (DPBS) (Mediatech, Inc., Herndon, VA). After 24 hrs, thymuses and spleens were removed, and mononuclear cells were

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prepared and stained with PE-Cy7-labeled CD4 (GK1.4), APC-labeled CD25 (7D4), and FITC-labeled anti-BrdU mAb using a commercial kit (BD Biosciences, San Jose, CA) according to the manufacturer's instructions. Samples were analyzed on a FACS Aria™ using Diva™ software.

#### Example 5.2: Results

[0243] Compared with those of control mice, the percentage of CD4<sup>+</sup>BrdU<sup>+</sup> cells from mice receiving IL-21R Fc was significantly increased, both in the thymus (FIG. 6, upper panel) and in the spleen (FIG. 6, lower panel), indicating that blockade of IL-21 leads to proliferation of CD4<sup>+</sup> T cells. The dot plots were generated after gating on lymphocytes (forward vs. side scatter). Data are from one representative experiment of three.

#### Example 6: Effects of IL-21 Blockade on Th1, Th2, and Th17 Responses

##### Example 6.1: Materials and Methods

[0244] Foxp3<sup>GFP/GFP</sup> mice (C57BL/6 (H-2<sup>b</sup>) background) expressing green fluorescent protein (GFP) knocked into a Foxp3 allele (Jung et al. (2000) *Mol. Cell. Biol.* 20:4106-14) were kindly provided by Dr. A. Rudensky, University of Washington, Seattle, WA. The murine MOG<sub>35-55</sub> peptide (MEVGWYRSPFSRVVHLYRNGK; SEQ ID NO:26) was from Bio Synthesis Inc. (Lewisville, TX).

[0245] Splenocytes were cultured in RPMI-1640 complete medium in 24-well plates at  $2 \times 10^6$  cells/well containing no stimuli, MOG<sub>35-55</sub> peptide (10 µg/ml), PLP<sub>139-151</sub> peptide (10 µg/ml), or Con A (5 µg/ml). After 3 days, cells were stimulated by PMA, ionomycin, and brefelmin A (Sigma-Aldrich, St. Louis, MO) for 5 hrs. Antibodies were directly labeled with one of the following fluorescent tags: FITC, PE, PerCP-Cy5.5, APC, Alexa-647, CD25 (7D4), CD4 (GK1.4), CD8 (53-6.7), IFN-γ (XMG1.2), IL-10 (JESS-16E3) (BD Biosciences, San Jose, CA), Foxp3 (FJK-16s) (eBioscience, San Diego, CA). Cells were harvested at the end of the incubation period and stained with a combination of anti-CD4-FITC, anti-

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CD8-PerCP-Cy5.5, and anti-CD25-PE. The cells were fixed and permeabilized using Cytofix/Cytoperm™ solution (BD Biosciences Pharmingen, San Diego, CA), and then stained with Alexa-647-conjugated IFN- $\gamma$  and IL-10 (BD Biosciences Pharmingen). Cells from Foxp3<sup>GFP/GFP</sup> mice were stained with PE-Cy7-labeled CD4 (GK1.4) and APC-labeled CD25 (7D4). For IL-21R expression, cells were stained with PE-labeled IL-21R Ab (eBio4A9) (eBioscience). Appropriate isotype controls were always included. Samples were analyzed on a FACS Aria™ using Diva™ software.

[0246] For real-time quantitative reverse transcriptase polymerase chain reaction (RT-PCR), total RNA was extracted from cell suspensions of CNS using TRIZOL (Invitrogen Life Technologies, Carlsbad, CA). The first strand cDNA of each sample was synthesized using a reverse transcription kit (Invitrogen Life Technologies). RT-PCR was performed using an ABI Prism 7900-HT sequence system (PE Applied Biosystems, Norwalk, CT) with the QuantiTect SYBR Green PCR kit (Qiagen, Valencia, CA) in accordance with the manufacturer's instructions. The following primers were used:

TNF- $\alpha$  (F): 5'-ATG AGA AGT TCC CAA ATG GC-3'; (SEQ ID NO:17)

(R): 5'-CTC CAC TTG GTG GTT TGC TA-3'; (SEQ ID NO:18)

IL-10 (F): 5'-ACA GCC GGG AAG ACA ATA AC-3'; (SEQ ID NO:19)

(R): 5'-CAG CTG GTC CTT TGT TTG AA-3'; (SEQ ID NO:20)

IL-17 (F): 5'-CCT CCA GAA TGT GAA GGT CA-3'; (SEQ ID NO:21)

(R): 5'-CTA TCA GGG TCT TCA TTG CG-3'; (SEQ ID NO:22)

HPRT (F): 5'-AGC CTA AGA TGA GCG CAA GT-3'; (SEQ ID NO:23)

(R): 5'-TTA CTA GGC AGA TGG CCA CA-3'. (SEQ ID NO:24)

The hypoxanthine-guanine phosphoribosyltransferase (HPRT) gene was amplified and served as an endogenous control. The first strand cDNA product was amplified with platinum Taq polymerase (Invitrogen Life Technologies) and gene-specific primer pairs. Each sample was assayed in triplicate and experiments were repeated twice. The relative amounts of mRNA were calculated by plotting the Ct (Cycle of



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Threshold), and average expression was determined by the comparative method ( $2^{-\Delta\Delta C_t}$ ); cytokine gene expression in naive spinal cord was set at 1. The data are expressed as mean  $\pm$  SEM.

#### Example 6.2: Results

[0247] One possible reason that the susceptibility to EAE increased after IL-21R Fc treatment is that immunization with PLP peptide induced pathogenic cytokine production. To test this possibility, the production of IL-10 and IFN- $\gamma$  in control mice and in mice receiving IL-21R Fc was quantified by intracellular cytokine staining. Single-cell suspensions of spleen were prepared from mice injected with IL-21R Fc or IgG2a Ab on day 12 after immunization. The expression of CD4 and CD8 vs. intracellular IFN- $\gamma$  by splenocytes was compared with an appropriate isotype control gate. The levels of IFN- $\gamma$ -producing cells were significantly higher in the IL-21R Fc-injected mice compared with those of control mice (**FIG. 7A**). Dot plots generated after gating on lymphocytes are shown. The data are from one representative of three experiments. Of note, IL-21R Fc-injected mice, which displayed severe clinical symptoms and CNS inflammation, showed a 2- and 8-fold increase of IFN- $\gamma$  production in CD4 $^{+}$  and CD8 $^{+}$  T cells, respectively. IL-21R Fc treatment did not, however, significantly alter IL-10 production by CD4 $^{+}$  and CD8 $^{+}$  T cells (data not shown). These results suggest that the blockade of IL-21 enables expansion of T cells producing IFN- $\gamma$  after immunization with PLP<sub>139-151</sub> peptide.

[0248] Recent studies have revealed that IL-17-producing T cells, rather than IFN- $\gamma$ -producing T cells, may be more important in the pathogenesis of EAE (Hofstetter et al. (2007) *J. Immunol.* 178:1372-78; Bettelli et al. (2006) *supra*). To determine which cytokines/T cells are important in the pathogenesis of EAE, the levels of TNF- $\alpha$  (Th1), IL-10 (Th2), and IL-17 mRNA in the CNS of IL-21R Fc-injected and control mice were quantified by RT-PCR. On day 12, mice were sacrificed and the CNS removed. As shown in **FIG. 7B**, levels of IL-17 and TNF- $\alpha$  were increased in

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the CNS of mice receiving IL-21R Fc as compared to controls ( $p<0.05$ ). The levels of IL-10 (**FIG. 7B**) and IL-4 (data not shown) were not significantly different compared to controls. These data suggest that increased IL-17 expression within the CNS may contribute to the pronounced inflammation and severity of EAE in IL-21R Fc-treated mice.

#### Example 7: IL-21 Blockade Affects Homeostasis of Tregs

##### Example 7.1: Materials and Methods

[0249] The number of Tregs expressing Foxp3 and the level of Foxp3 expression of the Tregs in IL-21-blocked and control mice was quantified. Cells from draining lymph nodes (LN) and spleens isolated on day 12 after PLP immunization of mice injected with either IL21R Fc or IgG2a Ab (control) were analyzed by flow cytometry for the expression of CD4 and CD25. The percentage of Tregs from IL-21-blocked mice was significantly decreased in the LN (**FIG. 8A**, upper panel) and spleen (**FIG. 8A**, lower panel) as compared to controls. The percentage of CD4<sup>+</sup> and CD25<sup>+</sup> T cells was gated on lymphocytes from individual mice. The dot plots are representative of three separate experiments.

##### Example 7.2: Results

[0250] In LN, the mean percentages of Tregs in mice receiving IL-21R Fc before and after immunization were  $5.00\pm0.52$  and  $4.75\pm0.35$ , respectively ( $p<0.05$  vs. control mice ( $7.10\pm0.31$ )) (**FIG. 8B**). In the spleen, the mean percentages of Tregs in mice receiving IL-21R Fc before and after immunization were  $3.46\pm0.72$  and  $3.23\pm0.62$ , respectively ( $p<0.05$  vs. control mice ( $5.82\pm0.50$ )) (**FIG. 8B**). Overall, IL-21R Fc-treated mice showed a significant decrease in the number of Tregs compared to control mice. Therefore, IL-21/IL-21R blockade reduced the percentage of Tregs cells in the LN and spleens of EAE mice.

[0251] The transcription factor Foxp3 is believed to have a central role in the activity and function of Tregs (e.g., Fontenot et al. (2003) *Nat. Immunol.* 4:330-36; Hori et al. (2003) *Science* 299:1057-61). Therefore, the expression of Foxp3 in

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relation to CD25 expression in CD4<sup>+</sup> cells was determined in EAE mice.

IL-21R Fc-treated mice had a 2-fold decrease in the percentages of Foxp3<sup>+</sup> in CD25<sup>+</sup>CD4<sup>+</sup> cells in the draining LN (**FIG. 8C**, upper panel) and spleen (**FIG. 8C**, lower panel) as compared to controls. The expression of Foxp3 in relation to CD25<sup>+</sup> cells was gated on CD4<sup>+</sup> cells. The dot plots are representative of three separate experiments.

[0252] In LN, the mean percentages of Foxp3 and CD25<sup>+</sup> CD4<sup>+</sup> cells in mice receiving IL-21R Fc before and after immunization were 6.60±0.32 and 7.90±0.25, respectively ( $p<0.05$  vs. control mice (11.10±0.41)) (**FIG. 8D**). In the spleen, the mean percentages of Foxp3 and CD25<sup>+</sup>CD4<sup>+</sup> cells in mice receiving IL-21R Fc before and after immunization were 11.46±3.72 and 10.23±3.62, respectively ( $p<0.05$  vs. control mice (18.24±1.67)) (**FIG. 8D**). The mean percentage of Foxp3 and CD25 double-positive cells was gated on CD4<sup>+</sup> cells.

[0253] Taken together, these data demonstrate that IL-21/IL-21R blockade reduces the frequency of Treg cells and expression of Foxp3 in EAE. These findings were confirmed by studies in Foxp3<sup>GFP/GFP</sup> mice, which showed that treatment with IL-21R Fc reduced Treg cell frequency and Foxp3 expression (**FIG. 8E**).

Splenocytes isolated on day 7 after treatment with either IL-21R Fc or IgG2a Ab were analyzed by flow cytometry for expression of CD4, CD25, and Foxp3. The percentage of CD4<sup>+</sup>CD25<sup>+</sup> T cells gated on lymphocytes from individual mice is shown in the upper panel. The percentage of Foxp3 and CD25<sup>+</sup> cells is shown in the middle panel. The expression of Foxp3 by CD4<sup>+</sup> cells in relation to CD25<sup>+</sup> cells gated on CD4<sup>+</sup> cells is shown in the lower panel. The dot plots are representative of three separate experiments.

**Example 8: Blockade of IL-21 Alters the Encephalitogenic Potential of T cells and Reduces Suppressive Activity of CD4<sup>+</sup>CD25<sup>+</sup> Cells**

**Example 8.1: Materials and Methods**

[0254] For adoptive transfer experiments, SJL/J (H-2<sup>S</sup>) mice were immunized with PLP<sub>139-151</sub> peptide/CFA/PT, as described above, and then injected with 250 µg of

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either IL-21R Fc or IgG2a Ab (control) two days before or seven days after immunization. Eleven days after immunization, draining LN cells were cultured for four days in 15% Click's EHAA medium at a density of  $2 \times 10^6$  cells/ml and stimulated with 20 ng/ml of IL-12 and 50  $\mu$ g/ml of PLP peptide. PLP-activated T cells ( $1 \times 10^7$ ) were injected intraperitoneally into recipient mice that had been irradiated with 350 rads 1 hr earlier (Bai et al. (2004) *J. Exp. Med.* 200:447-58). Clinical scores were checked daily on a scale of 1-5, with gradations of 0.5 for intermediate scores.

[0255] For isolation of Tregs, SJL/J (H-2<sup>S</sup>) mice were immunized with PLP<sub>139-151</sub> peptide/CFA/PT, as described above, and then injected with 250  $\mu$ g of either IL-21R Fc or IgG2a Ab (control) seven days after immunization. Eleven days after immunization, mononuclear cells were obtained by mincing spleens through a wire mesh (BD Bioscience). CD4<sup>+</sup>CD25<sup>+</sup> T cells were isolated using a mouse CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cell isolation kit (Miltenyi Biotec, Auburn, CA). Briefly, CD4<sup>+</sup> T cells were first isolated through negative selection by removing all other cell types. These CD4<sup>+</sup> T cells were then incubated with 10  $\mu$ l of magnetic beads conjugated with an anti-CD25 antibody to isolate CD4<sup>+</sup>CD25<sup>+</sup> T cell populations. Isolated T cells were >95% pure upon reanalysis by flow cytometry.

[0256] For cotransfer experiments, purified CD4<sup>+</sup>CD25<sup>+</sup> cells ( $3.4 \times 10^6$ ) from IL-21R Fc-treated or control mice, and PLP-activated encephalitogenic T cells ( $1.0 \times 10^7$ ) from EAE mice (1:3 ratio), were injected intraperitoneally into naive recipient mice that had been irradiated with 350 rads 1 hr earlier.

#### Example 8.2: Results

[0257] To test whether IL-21 blockade drives on encephalitogenic T cells (escaping from the suppressive effects of Tregs and becoming more aggressive), PLP<sub>139-151</sub>-specific T cells from control mice and IL-21R Fc-treated mice were isolated and transferred into naive recipients. As shown in **FIG. 9A**, mice receiving PLP-specific T cells from IL-21R Fc-treated mice developed more severe EAE as compared to recipients of cells from control mice. The mean clinical score of recipients receiving

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cells from mice injected with IL-21R Fc (day -2,  $2.32 \pm 0.44$ ; day +7,  $2.70 \pm 0.41$ ) was significantly higher than that of the controls ( $1.84 \pm 0.45$ ), regardless of the time of IL-21R Fc treatment ( $p < 0.05$  (day -2);  $p < 0.01$  (day +7)). Additionally, the recipients receiving cells from mice immunized on day -2 or day +7 had an earlier onset of disease ( $9.50 \pm 1.00$  and  $9.00 \pm 0.50$  days after transfer, respectively) compared to controls ( $11.00 \pm 0.53$  days after transfer). Therefore, IL-21 blockade induces more pathogenic T cells in EAE.

[0258] Tregs from controls and mice receiving IL-21R Fc were isolated and cotransferred with encephalitogenic T cells into recipients at a 1:3 ratio. As shown in **FIG. 9B**, Tregs from control mice significantly reduced disease severity in the recipients, whereas Tregs from IL-21R Fc-treated mice had only minimally protective effects. The mean maximal clinical score in recipients of Tregs from control mice was significantly lower than in recipients of Tregs from IL-21R Fc-treated mice ( $0.36 \pm 0.16$  vs.  $1.85 \pm 0.47$ , respectively,  $p < 0.001$ ). Therefore, Treg cells from IL-21/IL-21R-blockaded mice failed to suppress encephalitogenic cells.

#### Example 9: IL-21R<sup>-/-</sup> Mice Develop EAE Earlier and With More Neurological Impairment than Controls

##### Example 9.1: Materials and Methods

[0259] C57/BL6 mice were purchased from Taconic (Germantown, NY). IL-21R<sup>-/-</sup> mice were produced by known procedures (Collins et al. (2003) *Immunol. Res.* 28:131-40) and backcrossed to those of a B6 background for 10 generations.

[0260] EAE was induced by subcutaneous injections into the flank and tail base with 100  $\mu$ g of MOG<sub>35-55</sub> peptide emulsified in CFA containing 500  $\mu$ g of heat-inactivated *Mycobacterium tuberculosis* (Difco, Detroit, MI). Supplemental injections of 200 ng pertussis toxin were given intravenously on the same day and two days later (List Biologic, Campbell, CA). The mice were monitored daily for clinical signs of EAE and scored blind on a scale of 0 to 5 with graduations of 0.5 for intermediate scores (Mendel et al. (1995) *Eur. J. Immunol.* 25:1951-59): 0, normal; 1, limp tail or hind limb weakness but not both; 2, limp tail and hind-limb weakness

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or hind-limb paralysis; 3, complete hind-limb paralysis; 4, complete hind-limb paralysis with forelimb weakness or paralysis; 5, moribund or deceased state.

[0261] Mice were euthanized via inhalation of halothane (Sigma, St. Louis, MO) and fixed by cardiac perfusion with 4% paraformaldehyde. Spinal cords and brains were removed, cryoprotected in 20% sucrose solution in PBS, embedded in OCT (i.e., optimal cutting temperature medium) (Fisher Scientific, Waltham, MA), and then frozen for cryostat sectioning at 10  $\mu$ M. Lymphocytic infiltration and demyelination were evaluated by H&E and luxol fast blue (LFB) staining, respectively. Histological findings were graded into five categories: 0, no inflammatory cells; 1, leptomeningeal infiltration; 2, mild perivascular cuffing; 3, extensive perivascular cuffing; 4, extensive perivascular cuffing and severe parenchymal cell infiltration (Jee and Matsumoto (2001) *supra*).

[0262] Clinical scores were analyzed using the nonparametric Mann-Whitney *U* test. Comparisons between groups were evaluated by the one-tailed unpaired Student's *t* test for two groups, and ANOVA for multiple comparisons. Values of  $p < 0.05$  were considered significant.

#### Example 9.2: Results

[0263] To determine the influence of IL-21R deficiency on the development of EAE, EAE was induced with MOG<sub>35-55</sub> peptide in IL-21R<sup>-/-</sup> mice and control wild-type B6 mice. The clinical course of disease was monitored for more than 60 days. IL-21R<sup>-/-</sup> mice developed EAE with an earlier onset than did controls (12.5 days vs. 16.5 days, respectively,  $p < 0.05$ ) and with more severe disease symptoms (**FIG. 10A**, **Table 3**). The mean maximal disease scores for IL-21R<sup>-/-</sup> and control mice were 3.3 and 2.6, respectively (**FIG. 10A**, **Table 3**). Surprisingly, IL-21R<sup>-/-</sup> mice recovered from disease (as shown by a reduction in clinical score) faster than did control mice (**FIG. 10A**). However, by day 60 post-infection, there was no difference in severity of EAE between the two groups (**FIG. 10A**). Results are pooled from four independent experiments and are expressed as daily mean clinical scores.

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[0264] The extent of neurological damage in the two groups of mice was analyzed by histopathological analyses on spinal cords and brains. IL-21R<sup>-/-</sup> mice showed massive mononuclear cell infiltration and inflammation associated with demyelination, particularly at the peak phase of EAE (mean histological scores: 3.2±0.9 for IL-21<sup>-/-</sup> mice vs. 2.2±0.5 for control mice). Tissue inflammation was also higher during EAE recovery (mean histological scores: 1.5±0.8 for IL-21<sup>-/-</sup> mice vs. 0.5±0.1 for control mice) (**FIG. 10B** (arrows indicate regions of cellular infiltration and inflammation), **Table 3**). Collectively, these data suggest that the severity of EAE increases in the early phases of disease in the absence of IL-21R; recovery (reduction in clinical score), however, is more rapid.

**Table 3:** *MOG<sub>35-55</sub>-induced EAE in IL-21R<sup>-/-</sup> mice<sup>a</sup>*

Mouse Group	Clinical Status		Histological Score	
	Day of Onset (Mean ± SD)	Maximum Disease Score (Mean ± SD)	Peak	Recovery
B6 (control)	16.5 ± 6.2	2.6 ± 1.7	2.2 ± 0.5	0.5 ± 0.1
IL-21R <sup>-/-</sup>	12.5 ± 1.9*	3.3 ± 0.7*	3.2 ± 0.9 <sup>†</sup>	1.5 ± 0.5

<sup>a</sup> Groups of mice were immunized with MOG<sub>35-55</sub> and observed for 60-90 days thereafter. Sections of spinal cords and brains from representative EAE mice were stained with H&E for histological examination.

\* indicates  $p < 0.05$  and <sup>†</sup> indicates  $p < 0.01$  as compared to corresponding wild-type B6 controls.

#### Example 10: IL-21R Deficiency in EAE Associates with Increased Autoreactive T cell Responses

##### Example 10.1: Materials and Methods

[0265] Anti-NK1.1 mAb (PK136) (Koo and Peppard (1984) *Hybridoma* 3:301-03) was obtained from hybridomas from the American Type Culture Collection (Manassas, VA). Mouse IgG2a (Sigma, St. Louis, MO) was used as the isotype control antibody.

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[0266] For cell isolation and proliferation assays, the culture medium was complete RPMI 1640 containing 50 IU/ml penicillin, 50 µg/ml streptomycin, 2 mM L-glutamine, 10 mM HEPES, 10% FBS, 10 µM β-mercaptoethanol, and 1% nonessential amino acid (Mediatech, Inc., Herndon, VA). Mononuclear cells were obtained from spleens and prepared in single-cell suspensions as previously described (Vollmer et al. (2005) *supra*). Briefly, splenocytes were isolated mechanically by gentle scraping of fresh spleen tissues through a 70 µM strainer into RPMI 1640 with 1% FBS. Red blood cells were lysed with BD Pharm Lyse<sup>TM</sup> buffer (BD Bioscience), and then cells were washed in culture medium and filtered through a 40 µM strainer. Mononuclear cells ( $4 \times 10^5$ /well) were cultured in 96-well round-bottom plates with 200 µl culture medium in the presence of MOG<sub>35-55</sub> (10 µg/ml), Con A (5 µg/ml), or the indicated antigens for 72 hrs. Cell proliferation was assayed by incubation with 1 µCi [<sup>3</sup>H]thymidine/well (specific activity 42 Ci/mmol) (Amersham, Arlington Heights, IL) during the last 18 hrs of culture. Cells were harvested by an automatic cell harvester (TOMTEC, Hamden, CT) and the incorporated radioactivity was evaluated using a Wallac MicroBeta Counter (PerkinElmer, Waltham, MA).

[0267] For flow cytometry and CFSE labeling, aliquots of  $\sim 3 \times 10^7$  splenocytes were labeled with 0.5 µM CFSE at 37°C in a water bath for 10 min. Cells with or without CFSE labeling were cultured at 37°C for 3 days in 24-well plates ( $2 \times 10^6$  cells/well), with or without antigen (MOG<sub>35-55</sub> 10 µg/ml; or Con A 2.5 µg/ml), and then stimulated with PMA (20 ng/ml), ionomycin (1 µg/ml), and BFA (5 µg/ml) for 5 hrs at 37°C. Cells were then stained for surface markers with fluorochrome-conjugated mAbs for 15 min at 4°C: anti-CD3-PeCy5 (17A2), anti-CD4 ApcCy7 (GK1.5), anti-CD8α-PeCy7 (53-6.7), and anti-NK1.1-PE (PK136) (BD Pharmingen). After fixation and permeabilization, intracellular cytokines were stained using Alexa 647/PE-conjugated anti-IFN-γ, anti-IL-2, anti-IL-4, anti-IL-10, or anti-IL-17 mAb for 30 min at 4°C. For intracellular Foxp3 expression, fresh cells were stained with



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surface markers anti-CD4-FITC (H129.19) and anti-CD25 APC (PC61.5) for 15 min at 4°C, fixed, permeabilized, and then stained with anti-Foxp3-PE (FJK-16s) (eBioscience, San Diego, CA). Flow cytometric data were collected on a FACS Aria™ flow cytometer (Becton Dickinson, Mountain View, MD) and analyzed with Diva™ software. Isotype-matched negative control mAbs were used for all stains. To determine the percentage of cells producing the selected cytokines, values obtained with isotype controls were subtracted from those with specific mAbs.

[0268] For RT-PCR, total RNA was extracted from cell suspensions of spinal cords using TRIZOL (Invitrogen Life Technologies). First-strand cDNA of each sample was synthesized using a reverse transcription kit (Invitrogen Life Technologies, Carlsbad, CA). RT-PCR was performed as previously described (Lie et al. (2007) *J. Immunol.* 178:6227-35), using the following primers:

IL-17 (F): 5'-CCT CCA GAA TGT GAA GGT CA-3'; (SEQ ID NO:21)

(R): 5'-CTA TCA GGG TCT TCA TTG CG-3'; (SEQ ID NO:22)

IFN-γ (F): 5'-AGC TCT TCC TCA TGG CTG TT-3'; (SEQ ID NO:27)

(R): 5'-TTT GCC AGT TCC TCC AGA TA-3'; (SEQ ID NO:28)

HPRT (F): 5'-AGC CTA AGA TGA GCG CAA GT-3' (SEQ ID NO:23)

(R): 5'-TTA CTA GGC AGA TGG CCA CA-3'. (SEQ ID NO:24)

The HPRT gene was amplified and served as an endogenous control. One μl of first-strand cDNA product was amplified with platinum Taq polymerase (Invitrogen Life Technologies) and gene-specific primer pairs. Each sample was assayed in triplicate and experiments were repeated twice. The relative amounts of mRNA were calculated by plotting the Ct (Cycle of Threshold), and average relative expression was determined by the  $2^{-\Delta\Delta C_t}$  comparative method.

#### Example 10.2: Results

[0269] To determine the effects of IL-21R deficiency on T cell proliferation in EAE, autoreactive T cell responses in the absence of IL-21R were evaluated. Splenocytes from MOG<sub>35-55</sub>-immunized IL-21R<sup>-/-</sup> and control mice were harvested on the

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indicated days post-immunization and analyzed *ex vivo* for proliferative responses to MOG<sub>35-55</sub> (10  $\mu$ l) by CFSE dilution. Dot plots gated on lymphocytes were evaluated for percentages of proliferating cells. **FIG. 11** shows that the CD4<sup>+</sup> T cell response to MOG<sub>35-55</sub> in IL-21R<sup>-/-</sup> mice was higher than in controls in the early phases of disease, and waned by day 60 post-immunization. Results are representative of three independent experiments. These data suggest that lack of IL-21R affected autoreactive T cell proliferation during the initial course of EAE.

**[0270]** Production of cytokines was also measured. Splenocytes from MOG<sub>35-55</sub>-immunized IL-21R<sup>-/-</sup> and control mice were harvested on the indicated days post-immunization and cultured in the presence of MOG<sub>35-55</sub> (10  $\mu$ l) for 72 hrs.

Intracellular cytokines were examined by flow cytometry. Compared to controls, peripheral CD4<sup>+</sup> and CD8<sup>+</sup> T cells of IL-21R<sup>-/-</sup> mice had increased expression of IFN- $\gamma$  on days 3 (data not shown) and 12 (**FIG. 12A**) post-immunization.

Interestingly, IL-21R<sup>-/-</sup> CD8<sup>+</sup> cells secreted more IFN- $\gamma$  than CD4<sup>+</sup> cells (**FIG. 12A**).

Dot plots shown are representative of three separate experiments. However, on day 60 post-immunization (recovery phase), IFN- $\gamma$  levels in IL-21R<sup>-/-</sup> CD4<sup>+</sup> and CD8<sup>+</sup> cells were decreased and comparable to those of control mice (**FIG. 12B**). As reported previously (Liu et al. (2006) *supra*), the numbers and percentages of CD4<sup>+</sup> and CD8<sup>+</sup> cells were comparable in IL-21R<sup>-/-</sup> and control mice (data not shown).

Results shown are data pooled from three independent experiments.

**[0271]** During the peak of EAE (day 12 post-immunization), CD4<sup>+</sup> cells from IL-21R<sup>-/-</sup> mice also produced more IL-4 and IL-10 as compared to controls (**FIG. 12C**). Dot plots shown are representative of three separate experiments.

These results suggest that IL-21R-deficiency in EAE is associated with a temporary expansion of Th1 and Th2 responses (particularly Th1), an aspect that wanes over the course of the disease.

**[0272]** Recently, IL-17-producing T cells (Th17) have been implicated to play a prominent role in the pathogenesis of EAE (Bailey et al. (2007) *supra*). Yet, cytokine mRNA expression in the spinal cords of IL-21R<sup>-/-</sup> and control EAE mice

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was not statistically different (**FIG. 12D**). Total RNA was isolated from spinal cords of naive mice and mice on day 25 post-immunization and analyzed by RT-PCR. The results are represented as fold-increase as compared to naive controls, and are expressed as mean  $\pm$  SD. However, whereas there were no significant differences in IL-17 production in IL-21R<sup>-/-</sup> mice as compared to controls, IL-21R<sup>-/-</sup> EAE mice did show a trend toward lower IL-17 production (**FIG. 13**).

**Example 11: Recovery from EAE Associates with the Insurgence of T Regulatory (Treg) Cells in IL-21R<sup>-/-</sup> Mice**

**Example 11.1: Materials and Methods**

[0273] For Treg cell suppression assays, splenic CD4<sup>+</sup>CD25<sup>+</sup> or CD4<sup>+</sup>CD25<sup>-</sup> T cells were isolated using a mouse CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cell isolation kit (Miltenyi Biotec), according to the manufacturer's instructions. CD4<sup>+</sup> T cells were first isolated through negative selection by removing all other cell types. These isolated CD4<sup>+</sup> T cells were then incubated with magnetic beads conjugated with anti-CD25 antibody to separate CD4<sup>+</sup>CD25<sup>+</sup> and CD4<sup>+</sup>CD25<sup>-</sup> T cell populations. CD4<sup>+</sup>CD25<sup>+</sup> or CD4<sup>+</sup>CD25<sup>-</sup> T cells were then sorted using a FACS Aria<sup>TM</sup>. Isolated T cells were >95% pure upon reanalysis by flow cytometry. CD4<sup>+</sup>CD25<sup>-</sup> cells were used as responders. CD4<sup>+</sup>CD25<sup>-</sup> T cells ( $1 \times 10^5$ ) were cultured in U-bottom 96-well plates with  $10^4$  splenic DCs, MOG<sub>35-55</sub> (10  $\mu$ g/ml), and anti-CD3 (0.1  $\mu$ g/ml). CD11c<sup>+</sup> cells were isolated from splenocytes of EAE mice at the indicated time point and further loaded with MOG<sub>35-55</sub> peptide *ex vivo*. These DCs were more potent than DCs from naive mice in generating a measurable T cell response (Liu et al. (2005) *J. Immunol.* 175:7898-904). Proliferation was determined by incorporation of <sup>3</sup>H thymidine for the last 18 hrs of the culture.

[0274] CNS-infiltrating mononuclear cells were isolated as previously described (Liu et al. (2007) *J. Immunol.* 178:6227-35). Briefly, fresh brains and/or spinal cords were removed from mice, cut into small pieces, and digested in 10 mM Hepes/NaOH buffer containing 1 mg/ml of collagenase for 1 hr at 37°C. Tissues were homogenized with a syringe, filtered through a 70  $\mu$ M cell strainer to obtain a

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single cell suspension, and centrifuged. Cell pellets were resuspended in 30% percoll and centrifuged against 70% percoll. The cell monolayer between the 30% and 70% percoll interface was collected for staining.

#### Example 11.2: Results

[0275]  $CD4^+CD25^+$  Treg cells are involved in the maintenance of immune tolerance. Because IL-21R and IL-2R share a common  $\gamma$  chain, the structure of IL-21R has homologies with that of IL-2R (Leonard and Spolski (2005) *supra*). To test whether Treg cells might be affected by IL-21R deficiency, the number of Treg cells was quantified and their Foxp3 expression (a marker of a suppressive phenotype for these cells) was assessed. Splenocytes from MOG<sub>35-55</sub>-immunized IL-21R<sup>-/-</sup> and control mice were prepared on the indicated days after immunization, stained with anti-CD4 and anti-CD25 mAb, and then stained with intracellular anti-Foxp3 mAb, as described above. IL-21R<sup>-/-</sup> EAE mice showed decreased numbers of  $CD4^+CD25^+$  Treg cells as compared to controls during EAE induction (day 3 post-immunization) and at the peak stage of EAE (day 12 post-immunization) (FIGs. 14A, 14B). Dot plots were gated on lymphocytes in two to four representative experiments. Results of frequencies of individual cell populations were pooled from three independent experiments. These findings correlate with corresponding decreased percentages of Foxp3<sup>+</sup> cells (FIGs. 14C, 14D;  $CD25^+Foxp3^+$  cells on  $CD4^+$  gated subpopulation) (\* indicates  $p<0.05$ ; † indicates  $p<0.01$ ).

[0276] In *in vitro* suppression assays, Treg cells isolated from IL-21R<sup>-/-</sup> EAE mice at day 12 post-immunization had a reduced capacity to inhibit the proliferation of effector T cells as compared to Treg cells isolated from IL-21R<sup>-/-</sup> EAE mice at day 60 post-immunization (FIG. 15). Purified  $CD4^+CD25^+$  Tregs and responder  $CD4^+CD25^-$  cells ( $1 \times 10^5$ ) were cultured at a 1:4 ratio for 60 hrs with 10  $\mu$ g/ml MOG<sub>35-55</sub>-primed  $10^4$  APC. Baseline proliferation of responder cells was  $2800 \pm 233$  CPM. The data shown represent two separate experiments with similar outcomes ( $p<0.05$ ).

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[0277] As EAE progressed, the differences between IL-21R<sup>-/-</sup> mice and control mice in the numbers of Treg cells, as well as their expression of Foxp3, disappeared (FIG. 14). Consistent with these observations, flow cytometric analysis of mononuclear cell infiltration into brain and spinal cord showed that, on day 12 post-immunization, IL-21R<sup>-/-</sup> mice had decreased numbers of CD4<sup>+</sup>CD25<sup>+</sup> cells as compared to control mice (data not shown). Yet, on day 60 post-immunization (recovery from EAE), IL-21R<sup>-/-</sup> and control mice had comparable numbers of CD4<sup>+</sup>CD25<sup>+</sup> cells (data not shown). Thus, similar to the effect on autoreactive T cells, IL-21R deficiency had a transitory influence on Treg cells in EAE.

#### Example 12: Treg Cells of IL-21R<sup>-/-</sup> Mice Are Highly Sensitive to IL-2

##### Example 12.1: Materials and Methods

[0278] IL-21 and IL-2 ELISAs were performed using the DuoSet ELISA kit from R&D Systems (Minneapolis, MN) (Coquet et al. (2007) *supra*). Briefly, splenic mononuclear cells (4×10<sup>5</sup>/well) from IL-21R<sup>-/-</sup> and control mice (after spleen removal at indicated time points) were cultured with anti-CD3 and anti-CD28 mAbs for 72 hrs. Supernatants were harvested and analyzed for the presence of IL-21 or IL-2 by ELISA. 96-well microtiter plates were coated with IL-21 or IL-2 capture Ab at 4°C overnight. After blocking with 10% FBS, samples or standards were added and incubated for 2 hrs at room temperature. Plates were incubated with detection Ab for 2 hrs and then with Streptavidin-HRP for 30 min, followed by color development with TMB substrate reagent. Results were expressed as OD at 450 nm.

[0279] For *in vitro* IL-2 and IL-21 cultures, splenic mononuclear cells (4×10<sup>5</sup>/well) from IL-21R<sup>-/-</sup> and control mice were cultured with IL-2 (10 µg/ml) or IL-21 (1, 10, or 100 µg/ml) for 4 days at 37°C before harvest.

##### Example 12.2: Results

[0280] Quantification of the production of IL-2 and IL-21 in IL-21R<sup>-/-</sup> and control mice showed that IL-21 was detectable as early as day 3 post-immunization, whereas IL-2 was undetectable at this stage (FIGs. 16A, 16B). IL-21 increased further by

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day 12 post-immunization, a time when IL-2 was also detected (**FIGs. 16A, 16B**).

Data shown are from one of three independent experiments with similar results.

Because similar kinetics of IL-21 and IL-2 production were observed in both IL-21R<sup>-/-</sup> and control mice, the data suggest the possibility that lack of IL-21R negatively impacted the numbers of Treg cells during early EAE and, subsequently, production of IL-2 could positively impact Treg cell numbers.

[0281] To assess whether IL-21R<sup>-/-</sup> Treg cells could respond to IL-2, splenocytes of IL-21R<sup>-/-</sup> and control mice were isolated and cultured in the presence of IL-2.

Splenocytes from IL-21R<sup>-/-</sup> and control mice were cultured with IL-2 (10 µg/ml) for 4 days. Flow cytometry studies indicated that IL-21R<sup>-/-</sup> Treg cells expanded in the presence of IL-2 (**FIGs. 16C, 16D**). **FIG. 16C** shows CD4<sup>+</sup>CD25<sup>+</sup> cells from IL-2 cultures. **FIG. 16D** shows Foxp3 expression by CD4<sup>+</sup> cells (upper panel), and in relation to CD25<sup>+</sup> cells gated on CD4<sup>+</sup> cells (lower panel), from IL-2 cultures. Plots shown are representative of four independent experiments.

### Example 13: Effects of EAE on NK Cells and NKT Cells in IL-21R<sup>-/-</sup> Mice

#### Example 13.1: Materials and Methods

[0282] For depletion of NK1.1<sup>+</sup> cells *in vivo*, mice were injected intraperitoneally with either 100 µg anti-NK1.1 mAb or IgG2a (control Ab) on day -2 post-immunization. Every five days thereafter, 50 µg anti-NK1.1 mAb or IgG2a was injected until the termination of experiments (Vollmer et al. (2005) *supra*). Depletion of NK1.1<sup>+</sup> cells was confirmed by flow cytometry with anti-mouse NK1.1-PE (PK136) (BD Pharmingen, San Diego, CA), and was always >90%.

#### Example 13.2: Results

[0283] NK cells (NK1.1<sup>+</sup>CD3<sup>-</sup>) play an important role in EAE because they can suppress disease (Huang et al. (2006) *supra*; Zhang et al. (1997) *supra*). Activated NKT cells (NK1.1<sup>+</sup>CD3<sup>+</sup>) are also important regulatory cells in this disease (Singh et al. (2001) *J. Exp. Med.* 194:1801-11; Zajonc et al. (2005) *J. Exp. Med.* 202:1517-26). Therefore, NK and NKT cells in IL-21R<sup>-/-</sup> mice during EAE were

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examined. Splenocytes or CNS inflammatory cells from groups of MOG<sub>35-55</sub>-immunized IL-21R<sup>-/-</sup> and control mice were prepared on the indicated days after immunization, and stained with anti-NK1.1 and anti-CD3 mAb.

[0284] In early disease (day 3 post-immunization) IL-21R<sup>-/-</sup> mice had reduced numbers of splenic NK cells as compared to controls (3.4% vs. 7.3%, respectively) (FIGs. 17A, 17B). No difference was found at other time points post-immunization (FIG. 17B), indicating that NK cells, by day 3 post-immunization, had expanded only in control mice and not in IL-21R<sup>-/-</sup> mice (FIG. 17B). Dot plots are representative of two to four independent experiments ( $p < 0.05$ ). Results of frequencies of individual cell populations were pooled.

[0285] While an expansion of splenic NKT cells was observed both in IL-21R<sup>-/-</sup> and control mice (FIG. 17A), the number of infiltrating NKT cells in the CNS was relatively unaffected (FIG. 17C). In the CNS, IL-21R<sup>-/-</sup> mice but not control mice showed an accumulation of NK cells at later stages of EAE (FIG. 17C).

[0286] FIG. 17D shows the clinical course of EAE in IL-21R<sup>-/-</sup> mice treated with either IgG2a (control) or anti-NK1.1 mAb. Importantly, depletion of NK cells worsened EAE in IL-21R<sup>-/-</sup> mice (FIG. 17D). The results are representative of three independent experiments and are expressed as daily mean clinical scores ( $p < 0.05$ ). Thus, NK cell accumulation in the CNS during late stages of EAE in IL-21R<sup>-/-</sup> mice is associated with protection from EAE.

#### Equivalents

[0287] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents of the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

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WHAT IS CLAIMED IS:

1. A method of treating, ameliorating, or preventing an autoimmune disorder, inflammatory disorder, transplant/graft rejection, lymphopenia, or graft-versus-host disease in a mammalian subject, comprising administering to the subject an IL-21/IL-21R agonist selected from the group consisting of agonistic IL-21/IL-21R polynucleotides or fragments thereof, agonistic IL-21/IL-21R polypeptides or fragments thereof, agonistic anti-IL-21/IL-21R antibodies or fragments thereof, and agonistic small molecules, in an amount sufficient to increase the level and/or activity of a Treg cell or a population of Treg cells in the mammalian subject, thereby treating, ameliorating, or preventing the autoimmune disorder, inflammatory disorder, transplant/graft rejection, lymphopenia, or graft-versus-host disease in the mammalian subject.
2. The method of claim 1, wherein the IL-21/IL-21R agonist is an agonistic anti-IL-21R antibody or fragment thereof.
3. The method of claim 1, wherein the IL-21/IL-21R agonist is an agonistic anti-IL-21 antibody or fragment thereof.
4. The method of claim 1, wherein the IL-21/IL-21R agonist is an agonistic anti-IL-21R antibody or fragment thereof that is capable of binding to an IL-21R comprised of an amino acid sequence at least 90% identical to the amino sequence set forth in SEQ ID NO:2, wherein the IL-21R is capable of binding an IL-21.
5. The method of claim 1, wherein the autoimmune disorder is selected from the group consisting of multiple sclerosis, juvenile idiopathic arthritis, psoriatic arthritis, hepatitis C virus-associated mixed cryoglobulinemia, polymyositis, dermatomyositis, polyglandular syndrome type II, autoimmune liver disease, Kawasaki disease, myasthenia gravis, IPEX, type I diabetes, psoriasis, hypothyroidism, hemolytic anemia, thrombocytopenia, spondyloarthritis, Sjögren's syndrome, rheumatoid



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arthritis, inflammatory bowel disease, Crohn's disease, ulcerative colitis, eczema, gastritis, and thyroiditis.

6. The method of claim 1, wherein the inflammatory disorder is selected from the group consisting of contact hypersensitivity and atopic dermatitis.

7. The method of claim 1, wherein the mammalian subject is a human.

8. The method of claim 1, wherein the IL-21/IL-21R agonist is an agonistic IL-21 polypeptide or fragment thereof that comprises an amino acid sequence with at least 90% identity to the amino acid sequence set forth in SEQ ID NO:9, and wherein the agonistic IL-21 polypeptide or fragment thereof is capable of binding an IL-21R.

9. The method of claim 8, wherein the IL-21 polypeptide is an IL-21 polypeptide that comprises the amino acid sequence set forth in SEQ ID NO:9.

10. The method of claim 1, wherein the IL-21/IL-21R agonist is an agonistic IL-21R polypeptide or fragment thereof that comprises an amino acid sequence with at least 90% identity to the amino acid sequence set forth in SEQ ID NO:2, and wherein the agonistic IL-21R polypeptide or fragment thereof is capable of binding an IL-21.

11. The method of claim 10, wherein the IL-21R polypeptide is an IL-21R polypeptide that comprises the amino acid sequence set forth in SEQ ID NO:2.

12. A method of transplanting/grafting an organ, tissue, cell, or group of cells to a mammalian subject comprising the steps of:

(a) administering to the subject an IL-21/IL-21R agonist selected from the group consisting of agonistic IL-21/IL-21R polynucleotides or fragments thereof, agonistic IL-21/IL-21R polypeptides or fragments thereof, agonistic anti-IL-21/IL-21R antibodies or fragments thereof, and agonistic small molecules, in an amount sufficient to reduce the risk of transplant/graft rejection; and

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(b) transplanting/grafting the organ, tissue, cell, or group of cells to the subject,

wherein the transplanting/grafting step (b) occurs either before, during, or after the administering step (a).

13. The method of claim 12, wherein the organ, tissue, cell, or group of cells transplanted/grafted is selected from the group consisting of heart, kidney, liver, lung, pancreas, bone marrow, cartilage, cornea, neuronal tissue, and cells thereof.

14. A method of treating, preventing, or ameliorating transplant/graft rejection in a mammalian transplant/graft recipient comprising:

(a) detecting a symptom of transplant/graft rejection in a transplant/graft recipient; and

(b) administering to the transplant/graft recipient an IL-21/IL-21R agonist selected from the group consisting of agonistic IL-21/IL-21R polynucleotides or fragments thereof, agonistic IL-21/IL-21R polypeptides or fragments thereof, agonistic anti-IL-21/IL-21R antibodies or fragments thereof, and agonistic small molecules.

15. The method of claim 14, wherein the symptom of transplant/graft rejection is selected from the group consisting of inflammation, decreased organ function, signs of rejection in biopsy, and fibrosis.

16. A method of treating, preventing, or ameliorating a cancer or an infectious disorder in a mammalian subject, comprising administering to the subject an IL-21/IL-21R antagonist selected from the group consisting of antagonistic IL-21/IL-21R polynucleotides or fragments thereof, antagonistic IL-21/IL-21R polypeptides or fragments thereof, antagonistic anti-IL-21/IL-21R antibodies or fragments thereof, and antagonistic small molecules, in an amount sufficient to decrease the level and/or activity of a Treg cell or a population of Treg cells in the

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mammalian subject, thereby treating, ameliorating, or preventing the cancer or infectious disorder in the mammalian subject.

17. A method of decreasing the transcription of HIV in a mammalian subject, comprising administering to the subject an IL-21/IL-21R antagonist selected from the group consisting of antagonistic IL-21/IL-21R polynucleotides or fragments thereof, antagonistic IL-21/IL-21R polypeptides or fragments thereof, antagonistic anti-IL-21/IL-21R antibodies or fragments thereof, and antagonistic small molecules, in an amount sufficient to decrease the level and/or activity of Foxp3 in the mammalian subject, thereby decreasing the transcription of HIV in the mammalian subject.

18. The method of either claim 16 or 17, wherein the IL-21/IL-21R antagonist is an antagonistic anti-IL-21R antibody or fragment thereof.

19. The method of either claim 16 or 17, wherein the IL-21/IL-21R antagonist is an antagonistic anti-IL-21 antibody or fragment thereof.

20. The method of either claim 16 or 17, wherein the IL-21/IL-21R antagonist is an antagonistic anti-IL-21R antibody or fragment thereof that is capable of binding to an IL-21R comprised of an amino acid sequence at least 90% identical to the amino acid sequence set forth in SEQ ID NO:2, and wherein the IL-21R is capable of binding an IL-21.

21. The method of claim 16, wherein the cancer or infectious disorder is a cancer, and wherein the cancer is a solid tumor, a soft tissue tumor, or a metastatic lesion.

22. The method of claim 21, wherein the cancer is breast cancer, ovarian cancer, lung cancer, leukemia, lymphoma, melanoma, colorectal cancer, or renal cancer.

23. The method of claim 16, wherein the cancer or infectious disorder is an infectious disorder, and wherein the infectious disorder is caused by a bacterial, viral, or parasitic infection.

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24. The method of claim 23, wherein the infectious disorder is caused by HIV infection, *S. mansonii* infection, hepatitis infection, Epstein-Barr virus infection, *Borrelia* infection, JC virus infection, cytomegalovirus infection, Coxsackie virus infection, papilloma virus infection, or herpes virus infection.
25. The method of either claim 16 or 17, wherein the mammalian subject is a human.
26. The method of either claim 16 or 17, wherein the IL-21/IL-21R antagonist is an antagonistic IL-21R polypeptide or fragment thereof comprised of an IL-21R extracellular domain and an Fc immunoglobulin fragment.
27. The method of claim 26, wherein the antagonistic IL-21R polypeptide or fragment thereof is comprised of an amino acid sequence at least 90% identical to the amino acid sequence set forth in SEQ ID NO:14.
28. The method of claim 26, wherein the IL-21R extracellular domain comprises about amino acids 1-235, 20-235, 1-236, or 20-236 of SEQ ID NO:2.
29. A method of modulating the level and/or activity of a Treg cell or a population of Treg cells in a mammalian subject, comprising administering to the subject an IL-21/IL-21R agonist or an IL-21/IL-21R antagonist in an amount sufficient to modulate the level and/or activity of the Treg cell or population of Treg cells in the mammalian subject.
30. A method of modulating the level and/or activity of Foxp3 in a mammalian subject, comprising administering to the subject an IL-21/IL-21R agonist or an IL-21/IL-21R antagonist in an amount sufficient to modulate the level and/or activity of Foxp3 in the mammalian subject.
31. The method of either claim 29 or 30, wherein the mammalian subject is a human.

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32. The method of either claim 29 or 30, wherein the IL-21/IL-21R agonist or IL-21/IL-21R antagonist is an IL-21/IL-21R agonist selected from the group consisting of agonistic IL-21/IL-21R polynucleotides or fragments thereof, agonistic IL-21/IL-21R polypeptides or fragments thereof, agonistic anti-IL-21/IL-21R antibodies or fragments thereof, and agonistic small molecules.
33. The method of claim 32, wherein the IL-21/IL-21R agonist is an agonistic anti-IL-21R antibody or fragment thereof.
34. The method of claim 32, wherein the IL-21/IL-21R agonist is an agonistic anti-IL-21 antibody or fragment thereof.
35. The method of claim 32, wherein the IL-21/IL-21R agonist is an agonistic anti-IL-21R antibody or fragment thereof that is capable of binding to an IL-21R comprised of an amino acid sequence at least 90% identical to the sequence set forth in SEQ ID NO:2, and wherein the IL-21R is capable of binding an IL-21.
36. The method of claim 32, wherein the IL-21/IL-21R agonist is an agonistic IL-21 polypeptide or fragment thereof that comprises an amino acid sequence with at least 90% identity to the amino acid sequence set forth in SEQ ID NO:9, and wherein the agonistic IL-21 polypeptide is capable of binding an IL-21R.
37. The method of claim 36, wherein the agonistic IL-21 polypeptide is an agonistic IL-21 polypeptide that comprises the amino acid sequence set forth in SEQ ID NO:9.
38. The method of claim 32, wherein the IL-21/IL-21R agonist is an agonistic IL-21R polypeptide or fragment thereof that comprises an amino acid sequence with at least 90% identity to the amino acid sequence set forth in SEQ ID NO:2, and wherein the agonistic IL-21R polypeptide or fragment thereof is capable of binding an IL-21.

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39. The method of claim 38, wherein the agonistic IL-21R polypeptide is an agonistic IL-21R polypeptide that comprises the amino acid sequence set forth in SEQ ID NO:2.

40. The method of either claim 29 or 30, wherein the IL-21/IL-21R agonist or IL-21/IL-21R antagonist is an IL-21/IL-21R antagonist selected from the group consisting of antagonistic IL-21/IL-21R polynucleotides or fragments thereof, antagonistic IL-21/IL-21R polypeptides or fragments thereof, antagonistic anti-IL-21/IL-21R antibodies or fragments thereof, and antagonistic small molecules.

41. The method of claim 40, wherein the IL-21/IL-21R antagonist is an antagonistic anti-IL-21R antibody or fragment thereof.

42. The method of claim 40, wherein the IL-21/IL-21R antagonist is an antagonistic anti-IL-21 antibody or fragment thereof.

43. The method of claim 40, wherein the IL-21/IL-21R antagonist is an antagonistic anti-IL-21R antibody or fragment thereof that is capable of binding to an IL-21R comprised of an amino acid sequence at least 90% identical to the sequence set forth in SEQ ID NO:2, and wherein the IL-21R is capable of binding an IL-21.

44. The method of claim 40, wherein the IL-21/IL-21R antagonist is an antagonistic IL-21R polypeptide or fragment thereof that is comprised of an IL-21R extracellular domain and an Fc immunoglobulin fragment.

45. The method of claim 44, wherein the antagonistic IL-21R polypeptide or fragment thereof is comprised of an amino acid sequence at least 90% identical to the sequence set forth in SEQ ID NO:14.

46. The method of claim 44, wherein the IL-21R extracellular domain comprises about amino acids 1-235, 20-235, 1-236, or 20-236 of SEQ ID NO:2.

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47. A method for increasing the ability of a vaccine composition containing an antigen to elicit a protective immune response against the antigen in a mammalian subject, comprising administering to the mammalian subject, either simultaneously with or sequentially to the vaccine composition, an effective adjuvanting amount of an IL-21/IL-21R antagonist, such that the ability of the vaccine composition to elicit the protective immune response is increased.
48. The method of claim 47, wherein the antigen is derived from a pathogen selected from the group consisting of a virus, bacterium, and protozoan.
49. The method of claim 47, wherein the IL-21/IL-21R antagonist is selected from the group consisting of antagonistic IL-21/IL-21R polynucleotides or fragments thereof, antagonistic IL-21/IL-21R polypeptides or fragments thereof, antagonistic anti-IL-21/IL-21R antibodies or fragments thereof, and antagonistic small molecules.
50. The method of claim 49, wherein the IL-21/IL-21R antagonist is an antagonistic anti-IL-21R antibody or fragment thereof.
51. The method of claim 49, wherein the IL-21/IL-21R antagonist is an antagonistic anti-IL-21 antibody or fragment thereof.
52. The method of claim 49, wherein the IL-21/IL-21R antagonist is an antagonistic anti-IL-21R antibody or fragment thereof that is capable of binding to an IL-21R comprised of an amino acid sequence at least 90% identical to the sequence set forth in SEQ ID NO:2, and wherein the IL-21R is capable of binding an IL-21.
53. The method of claim 47, wherein the mammalian subject is a human.
54. The method of claim 49, wherein the IL-21/IL-21R antagonist is an antagonistic IL-21R polypeptide or fragment thereof that is comprised of an IL-21R extracellular domain and an Fc immunoglobulin fragment.

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55. The method of claim 54, wherein the antagonistic IL-21R polypeptide or fragment thereof is comprised of an amino acid sequence at least 90% identical to the amino acid sequence set forth in SEQ ID NO:14.
56. The method of claim 54, wherein the IL-21R extracellular domain comprises about amino acids 1-235, 20-235, 1-236, or 20-236 of SEQ ID NO:2.
57. The method of claim 47, wherein the antigen is derived from a cancer or a tumor.
58. The method of claim 57, wherein the antigen is expressed on the surface of a cancer cell or a tumor cell.
59. A pharmaceutical composition useful as a vaccine, comprising an antigen from a pathogenic microorganism selected from the group consisting of a viral, bacterial, and parasitic microorganism, and an effective adjuvanting amount of an IL-21/IL-21R antagonist, in a pharmaceutically acceptable carrier.
60. A pharmaceutical composition comprising a cancer cell or tumor cell antigen in combination with an effective adjuvanting amount of an IL-21/IL-21R antagonist, in a pharmaceutically acceptable carrier.
61. The method of any one of claims 16, 17, 40, 47, 59, and 60, further comprising administering to the subject at least one additional therapeutic agent selected from the group consisting of cytokine inhibitors, growth factor inhibitors, immunosuppressants, anti-inflammatory agents, metabolic inhibitors, enzyme inhibitors, cytotoxic agents, and cytostatic agents.
62. The method of claim 61, wherein the at least one additional therapeutic agent is selected from the group consisting of TNF antagonists, anti-TNF agents, IL-12 antagonists, IL-15 antagonists, IL-17 antagonists, IL-18 antagonists, IL-22 antagonists, T cell-depleting agents, B cell-depleting agents, cyclosporin, FK506,



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CCI-779, etanercept, infliximab, rituximab, adalimumab, prednisolone, azathioprine, gold, sulphasalazine, hydroxychloroquine, minocycline, anakinra, atabcept, methotrexate, leflunomide, rapamycin, rapamycin analogs, Cox-2 inhibitors, cPLA2 inhibitors, NSAIDs, p38 inhibitors, antagonists of B7.1, B7.2, ICOSL, ICOS and/or CD28, and agonists of CTLA4.



1 human IL-21R 50  
 ---MPRGWAA PLLLLL..LQ GCWG..... CPDLVCYTDY LQTVCILEM  
 ---MPRGVA ALLLLL..LH GAWG..... CLDLTCYTDY LWTVCULET  
 human IL-2R $\beta$  MAAPALSMRL PLLLLLPLA TSWASAAVNG TSQFTCFYNS RANISCVWSQ 100  
 51  
 human IL-21R WN..LHPSTL TLTWQDQVEE LKDEATCSL HPSAHNATHA TYTCHM.....  
 mouse IL-21R RS..PNPSIL SLTWQDEYEE LQDQETFCSL HRSGHNTTHI WYTCHM.....  
 human IL-2R $\beta$  DGALQDTSCQ VHAWPDR...RRWNQTCEL....LPVSQA SWACNLILGA 150  
 101  
 human IL-21R .DVHEMADD LFSVNITDQS GN..YSQECG SELLAESIKP APPENVTVTF  
 mouse IL-21R .RLSQELSD E VFINVITDQS GN..NSQECG SFVLAESIKP APPLNVTIVAF  
 human IL-2R $\beta$  PDSQKLTIVD IVTLRVLCRE GVRWRVMAIQ DEKPFENLRL MAPISLQVNH 200  
 151  
 human IL-21R ..SGQYNISW RSDYEDAFY MLKGKLOVEL QYRNRGDPWA VSPRKLISV  
 mouse IL-21R ..SGRYDISW DSAYDEPSNY VLRGKLOVEL QYRNLRDPYA VRPVTKLISV  
 human IL-2R $\beta$  VETHRCNISW E..ISQASHY FER.HLEFEA RYLSPGHTWE EAP...LLTL 250  
 201  
 human IL-21R DSRVSILLPL EFRKDSYEL QVRAGMPGS SYQGTWSEWS DPVIFQTS.  
 mouse IL-21R DSRNVSILLPE RFHKDSSVOL QVRAAPQPGT SFRGTWSEWS DPVIFQTOA.  
 human IL-2R $\beta$  KQKQEWICLE TLTPTDQVEF QVRVKPIQGE F..TTWSPWS QPIAFRTKPA 300  
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 mouse IL-21R ..GEPRAGWD PHMLLLL...AVLLIVL.VF MGLKIHLPWR LMKKIWAPVP  
 human IL-2R $\beta$  ALGKDTIPWL GHLIVGISGA FGPTIIVYIL INCRNTGPM. LAKKVLKCNTP 350  
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 human IL-21R PRSPAKRIQL TELQEP.A.E LVESDGVKPK SPW...PTAO NSGGSAYSEE  
 mouse IL-21R SLXPAKAKKF PGLPGL.E..E QLECDGNSEP GHWCIIPLAA GOAVSAISEE  
 human IL-2R $\beta$  TQLLLQDDKV PEPASLSSNH SLTSCFTNQG YFFHFLPDAL ETEACQVYTF 450  
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 human IL-21R RDRPYGLVSI DTVTVLDAG PC...TWPCS CEDDGYPALD LDAGLESPG  
 mouse IL-21R RDRPYGLVSI DTVTVGDAEG LC...VWPCS CEDDGYPAMN LDAGRESGPN  
 human IL-2R $\beta$  YD.PYSEEDP DEGVAGAPTG SSPOPLPLS GEDDAYCTF. ....PS 500  
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 human IL-21R LEDPLLDAGT TVLSCGCVSA GSPGLGGPIG SLLDRLEKPL AD..GEDWAG  
 mouse IL-21R SEDLLIVTDF AFLSCGCVSG SGLRLGSGPG SILLDRLRISF AK..EGDWTA  
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 human IL-21R GLPWGGRSPG GVSESEAGSP LAGLMDTDF SGFVGSDCSS PVECDFTSPG  
 mouse IL-21R DPTWRIGSPG GGSESEAGSP .PGLMDTDF SGFAGSDGGS PVET.....  
 human IL-2R $\beta$  Q.PLGPPTPG VPDIVDFQPP F...ELVIRE AGEVVPDAG. PRE.GVSFFW 588  
 551  
 human IL-21R DEGPRSYLR QWVVIPLPLS SPGPQAS'---  
 mouse IL-21R DEGPRSYLR QWVVRTPPV DSGAQSS---  
 human IL-2R $\beta$  SRPGQGEFR ALNARUPLNT DAYLSLQELQ QODFTHLV

Fig. 2

Fig. 3

mIL-21		1	50
Translation of rat IL-21 CDS	(1)	-----MERTLVCLVVFIFLGTVAHKSSPQGPDRLLIRLRHLIDIVEQLK	
Translation of hIL-21	(1)	-----MERTLVCLILIFLGTVAHKSSPQRPDHLIRLRHLMDIVEQLK	
Consensus	(1)	MRSSPGNMERIVICLMVIFLGTLVHKSSSQGQDRHMIRMRLQIDIVDQLK	
	(1)	MERTLVCLIVIFLGTVAHKSSPQGPDRLLIRLRHLIDIVEQLK	
mIL-21		51	100
Translation of rat IL-21 CDS	(44)	IYENDLDPELLSAPQDVKGHCHEAFAFCQAKLKPSNPGNNKTFIIDLV	
Translation of hIL-21	(44)	IYENDLDPELLTAPQDVKGQCEHEAFACFQAKLKPSNTGNNKTFINDLL	
Consensus	(51)	NYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQLKSANTGNNERIINVSI	
	(51)	IYENDLDPELLSAPQDVKGNCHEAFAFCFQAKLKPSNTGNNKTFINDLI	
mIL-21		101	150
Translation of rat IL-21 CDS	(94)	AQLRRRLPARRGGKKQKHIAKCPSCDSYEKRTPKFEFLERLKWLLQKMIHQ	
Translation of hIL-21	(94)	AQLRRRLPAKRRTGNKQRHMAKCPSCDLYEKKTPKEFLERLKWLLQKMIHQ	
Consensus	(101)	KKLKRKPPSTNAGRROKHRLTCPCSDSYEKKPPKEFLERFKSLLQKMIHQ	
	(101)	AQLRRRLPAKRAGKKQKHIAKCPSCDSYEKKTPKEFLERLKWLLQKMIHQ	
mIL-21		151	162
Translation of rat IL-21 CDS	(144)	HLS-----	
Translation of hIL-21	(144)	HLS-----	
Consensus	(151)	HLSRTHGSEDS	
	(151)	HLS	

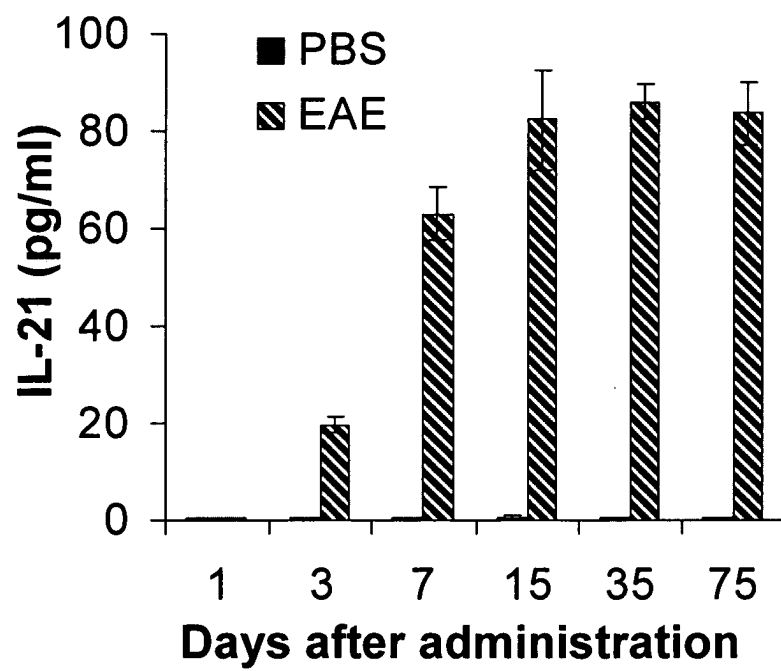
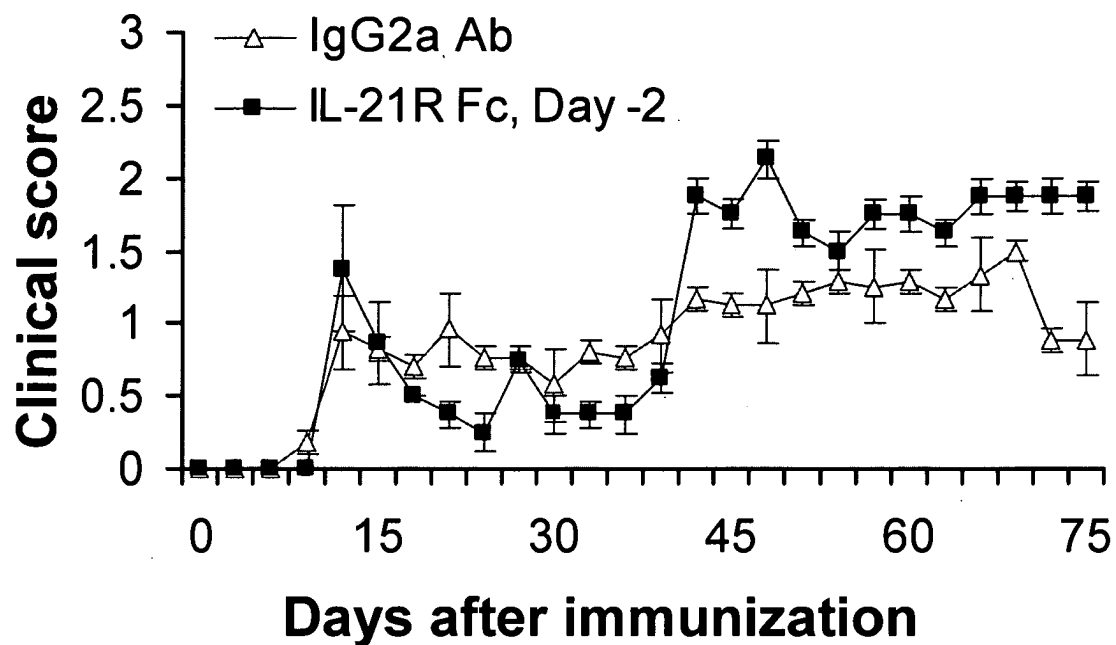
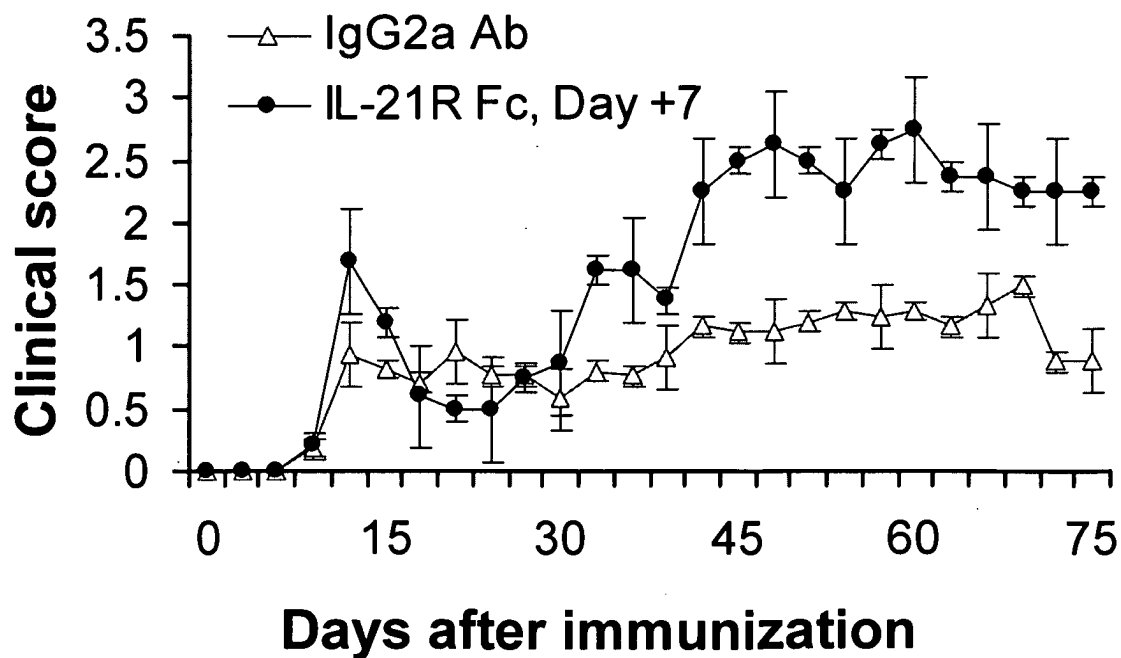
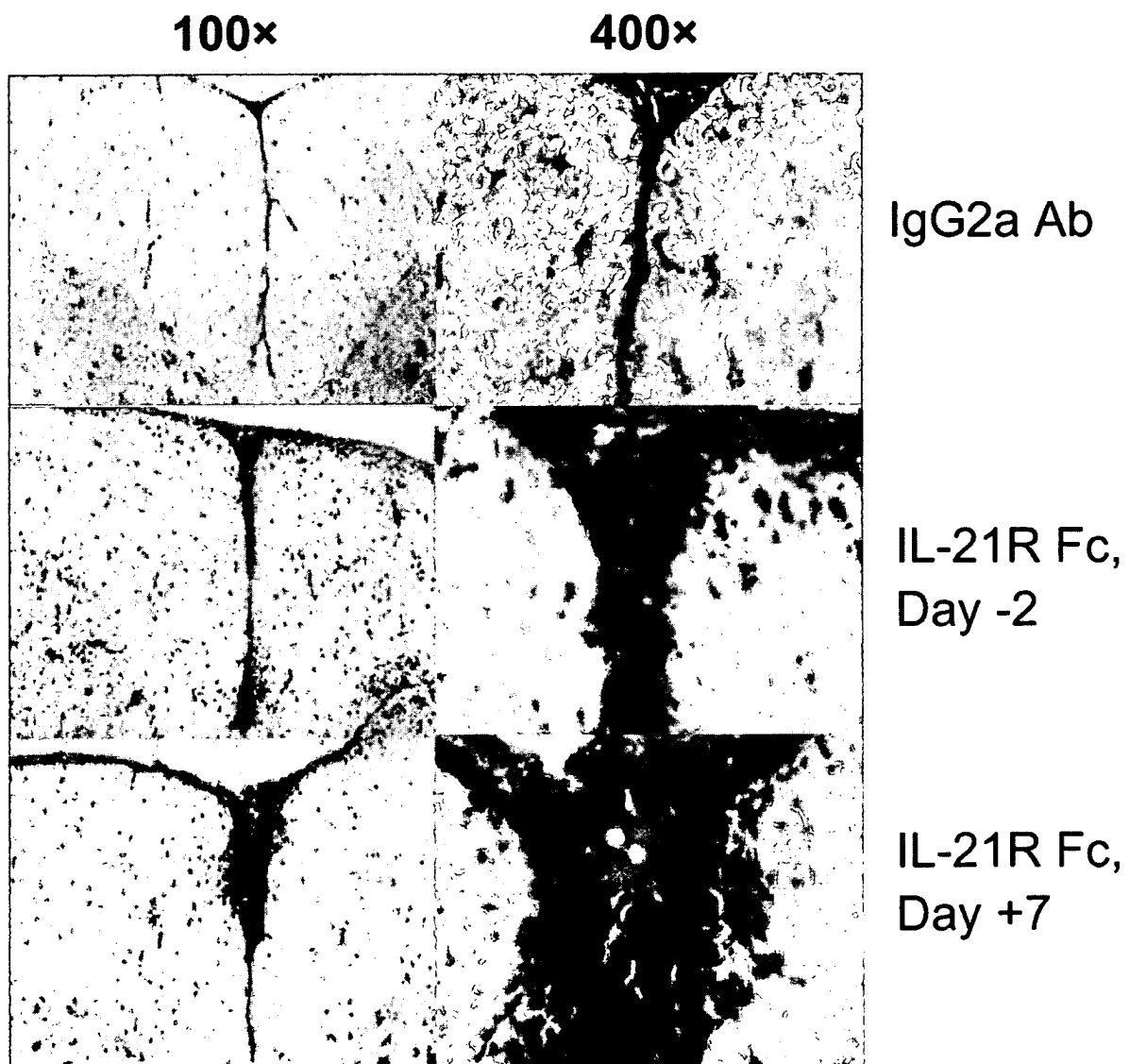


Fig. 4

**Fig. 5A****Fig. 5B**

**Fig. 5C**

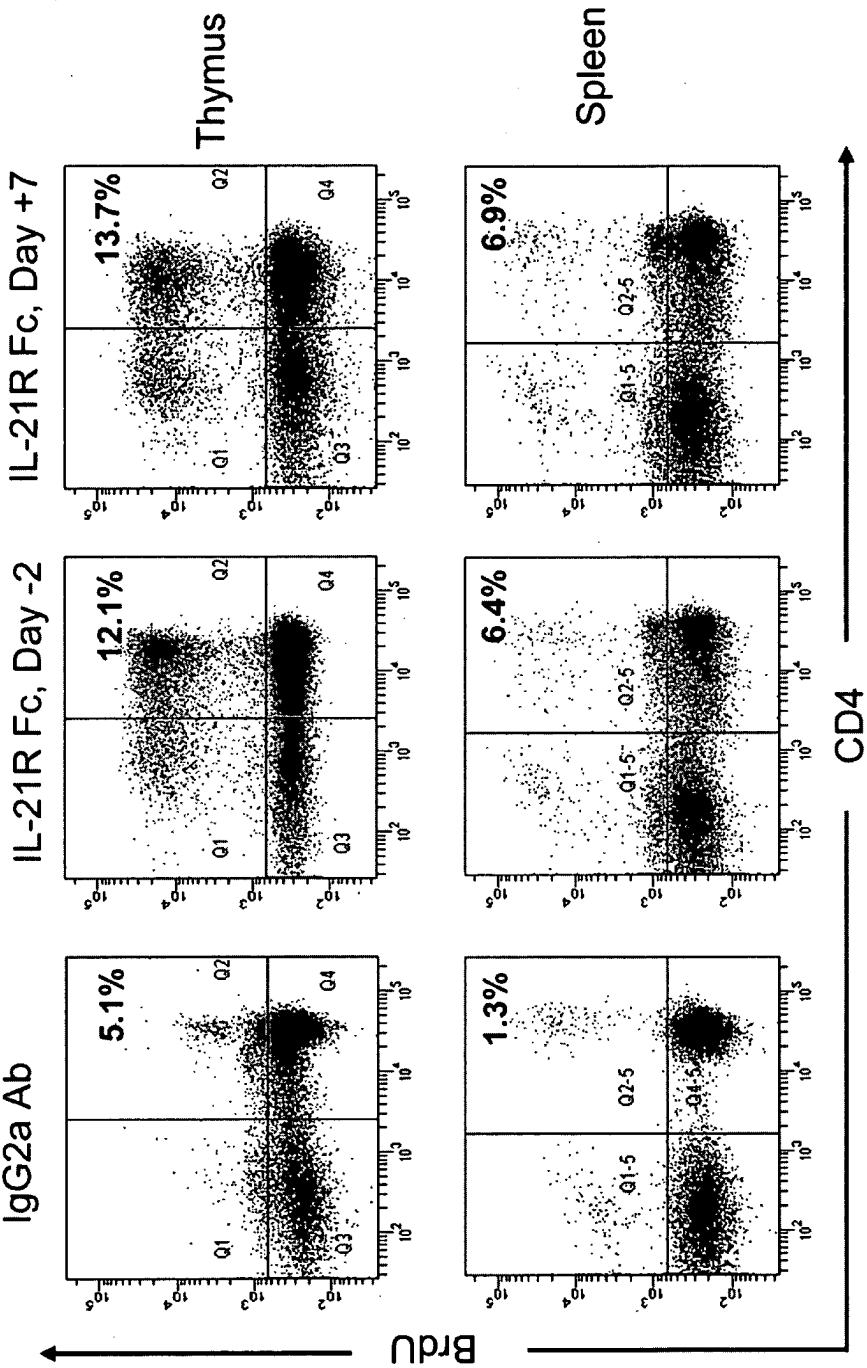
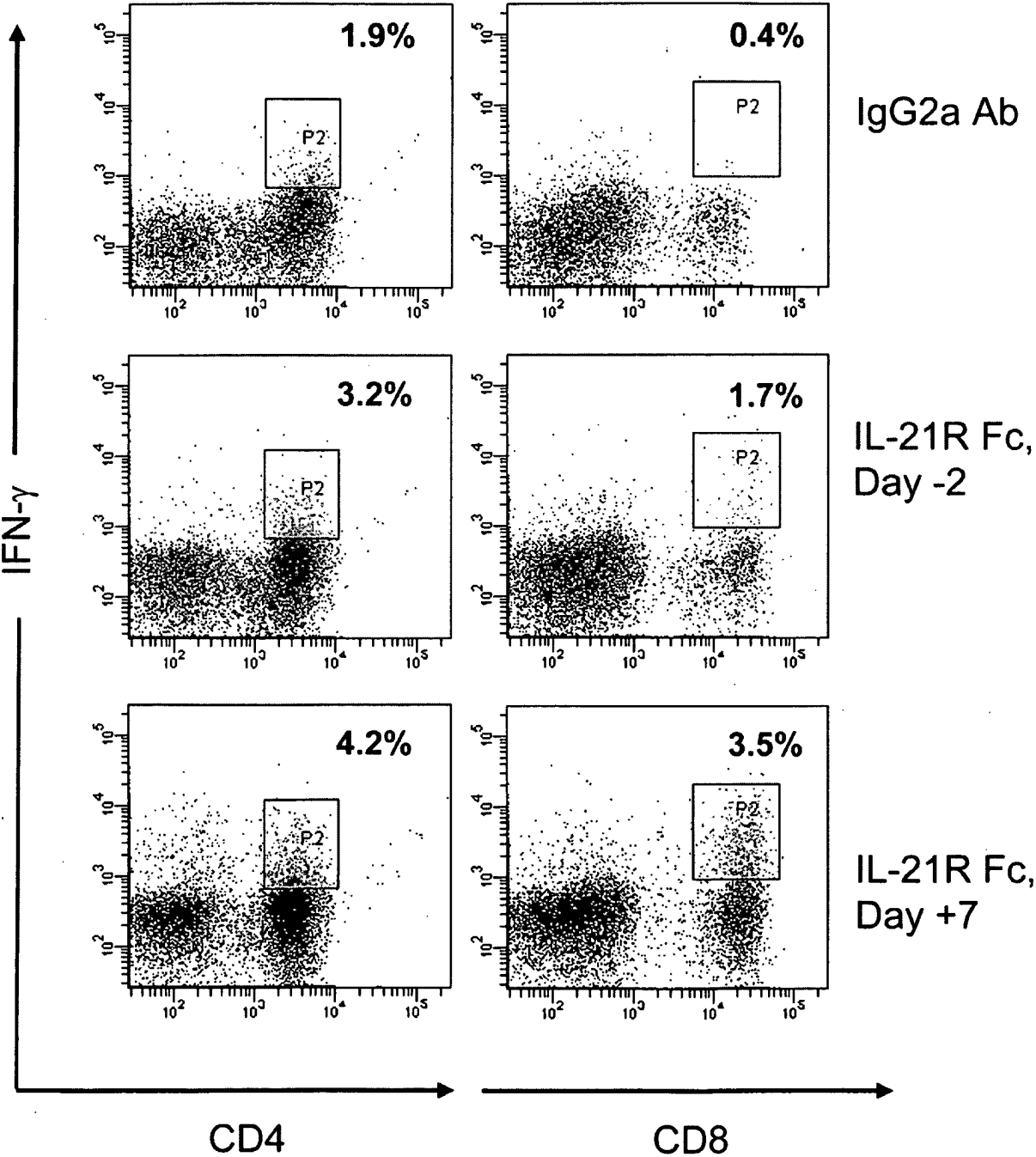
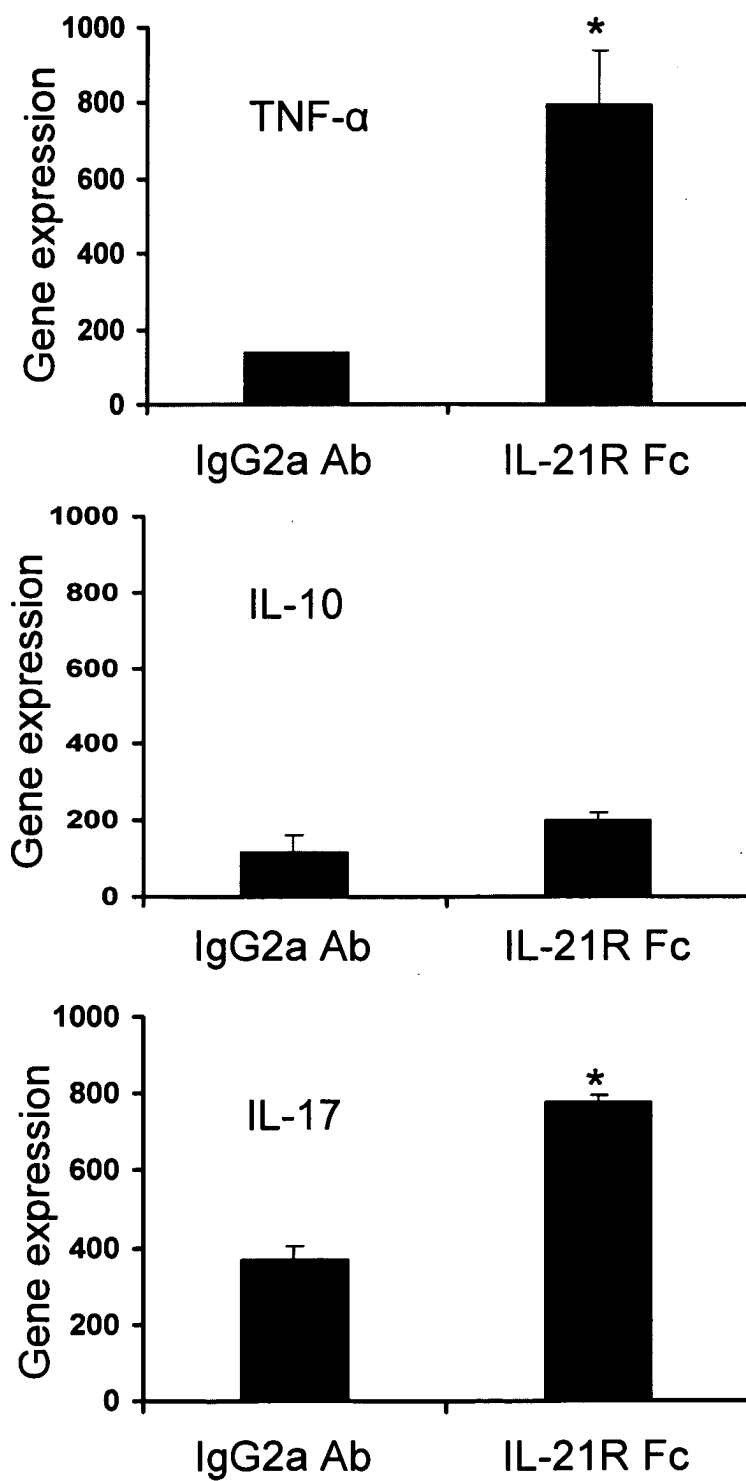


Fig. 6

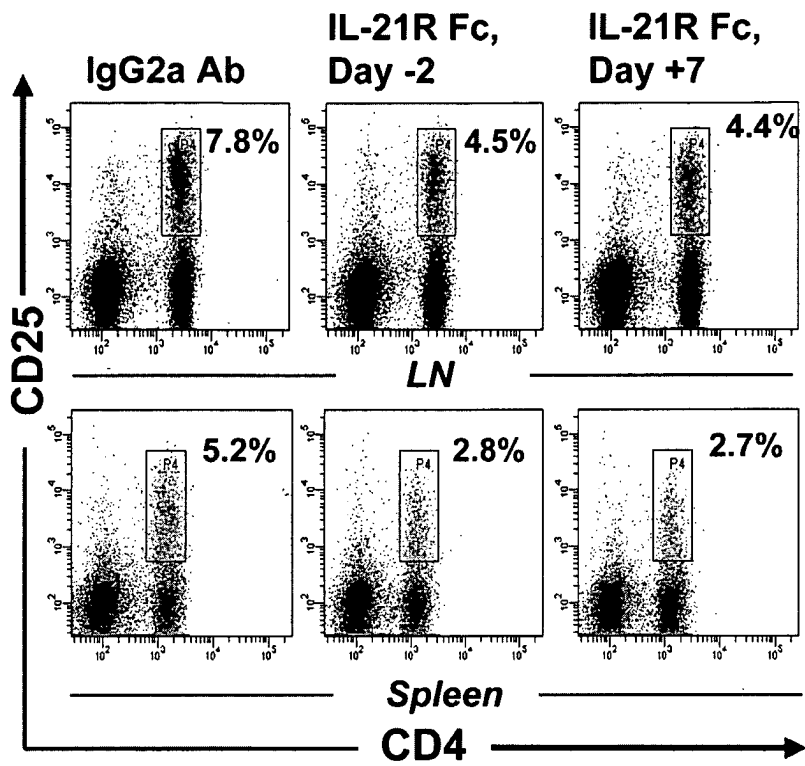


Fig. 7A



**Fig. 7B**

**Fig. 8A**



**Fig. 8B**

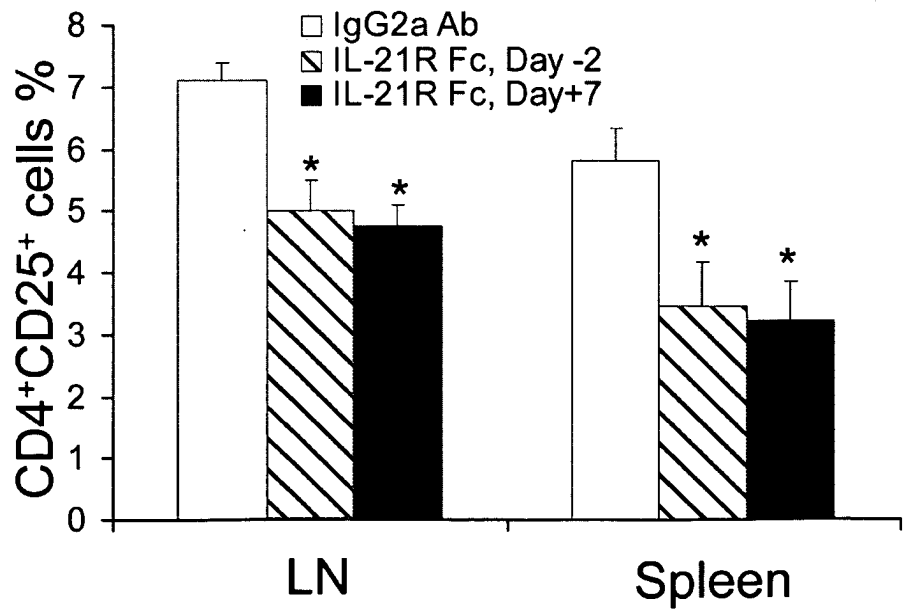


Fig. 8C

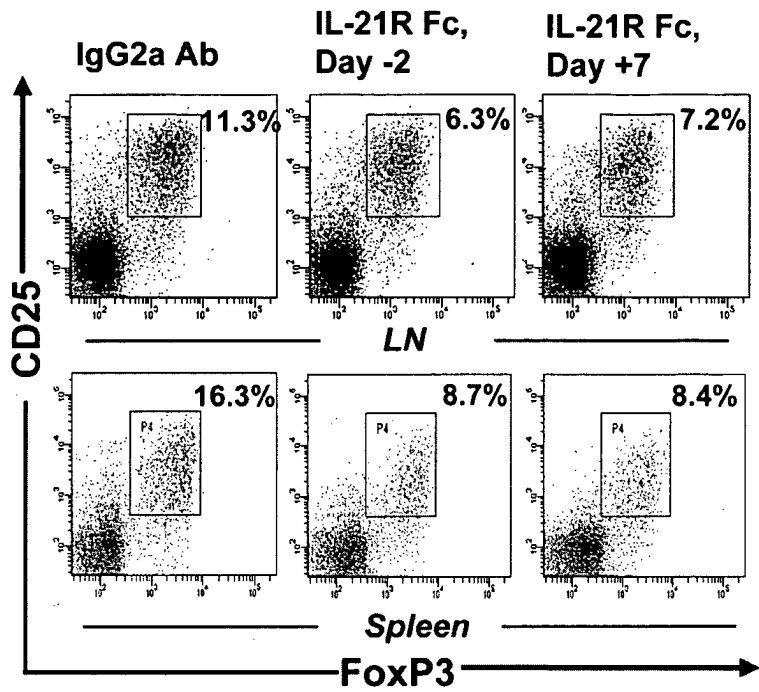


Fig. 8D

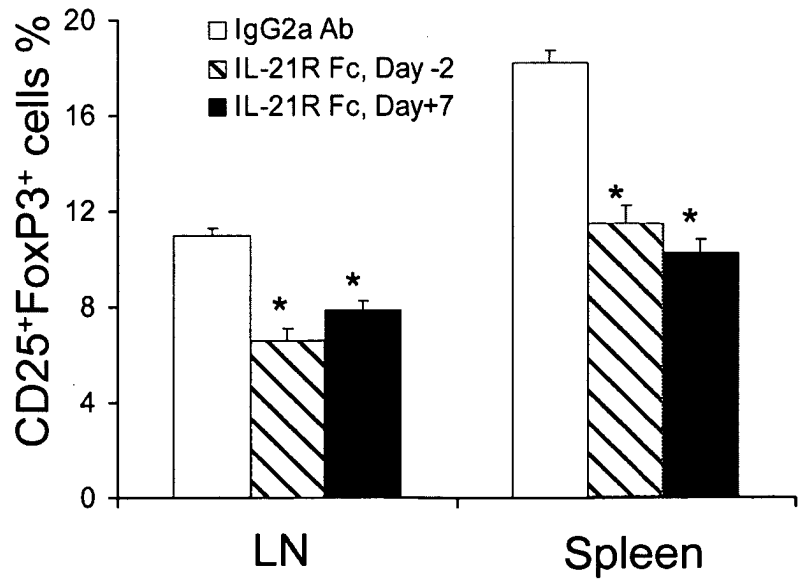
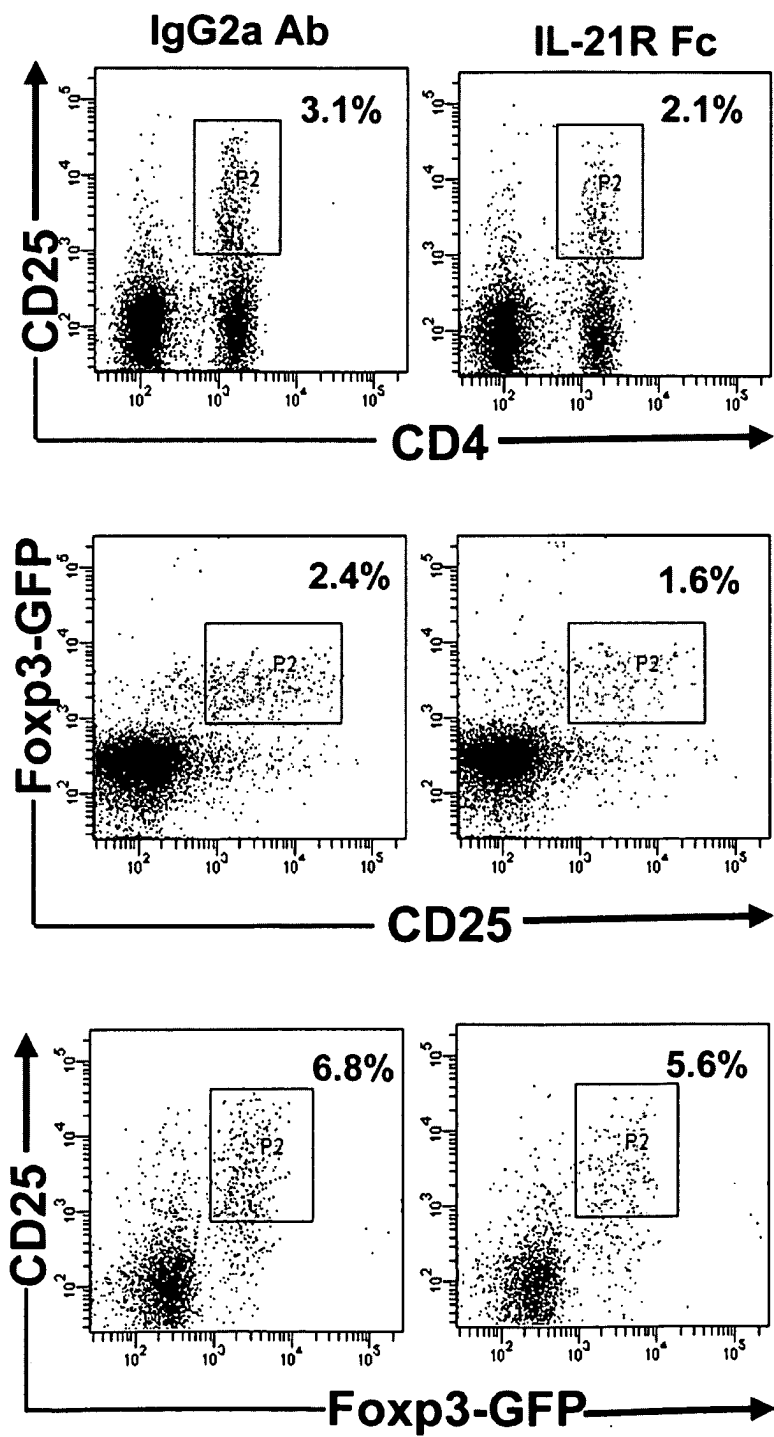


Fig. 8E



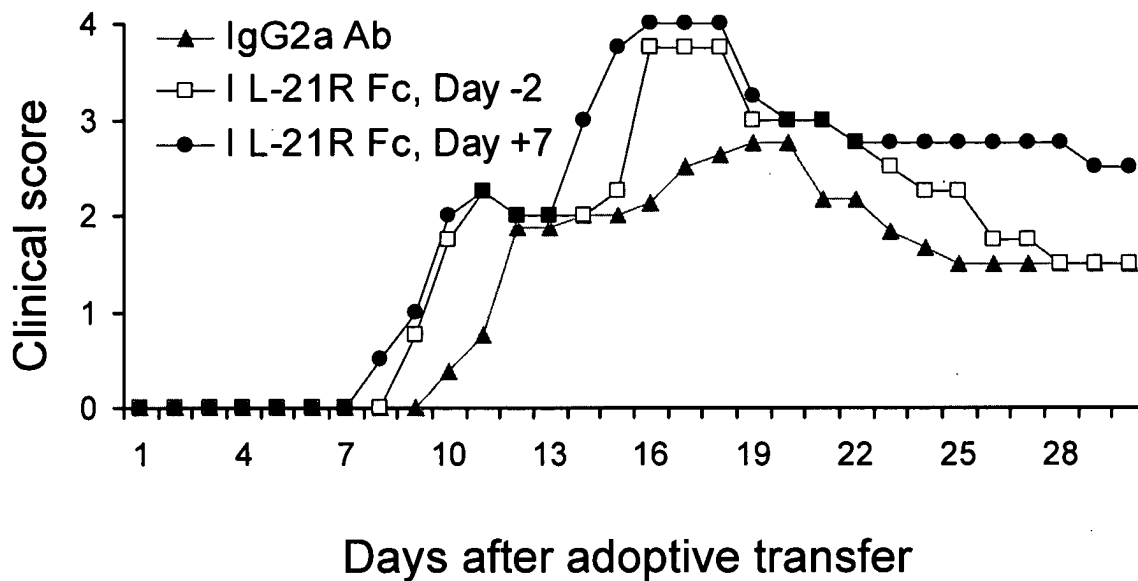
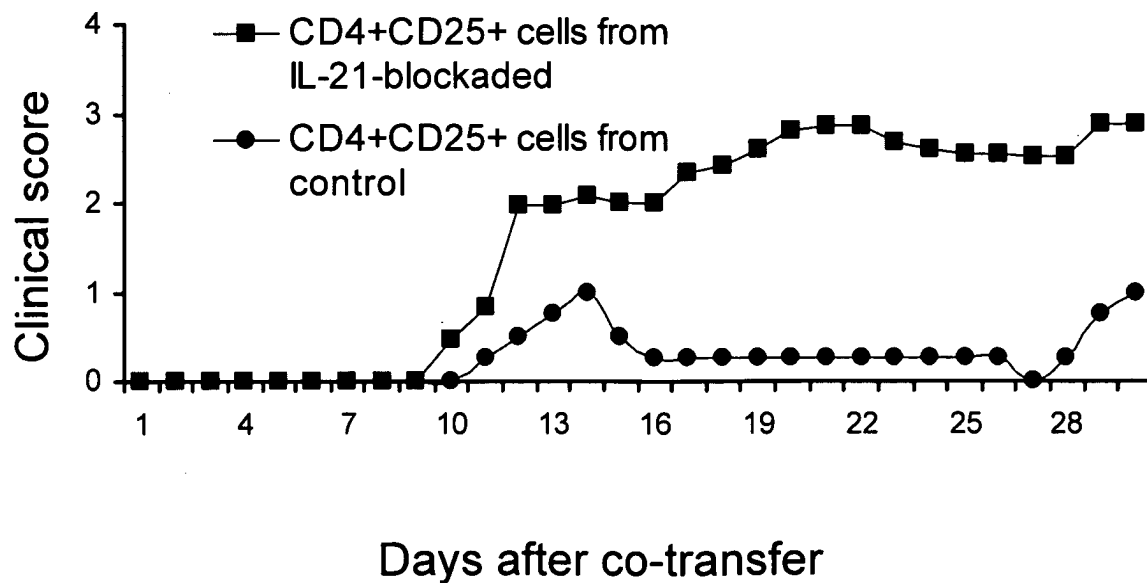
**Fig. 9A****Fig. 9B**

Fig. 10A

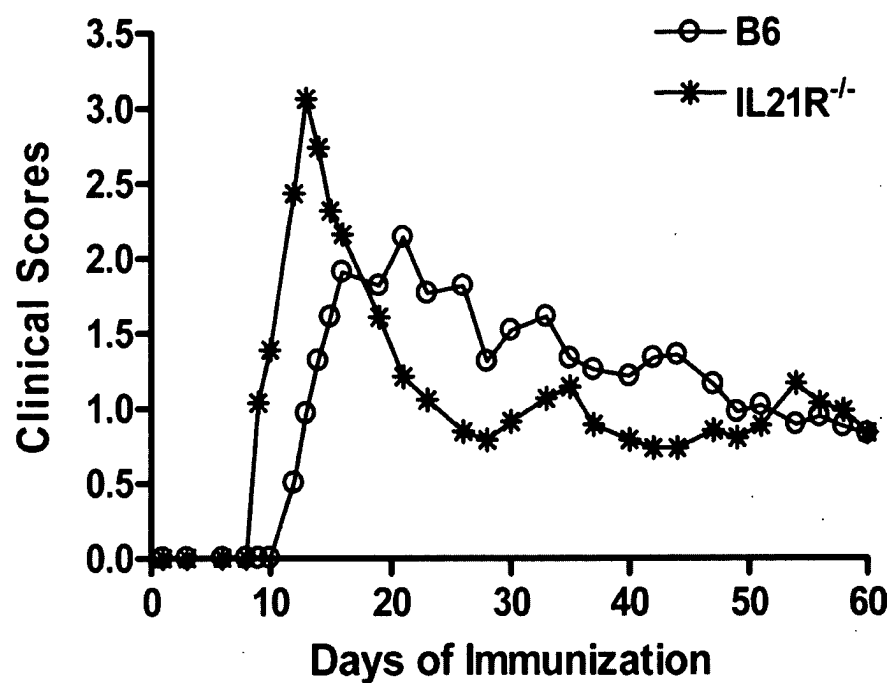
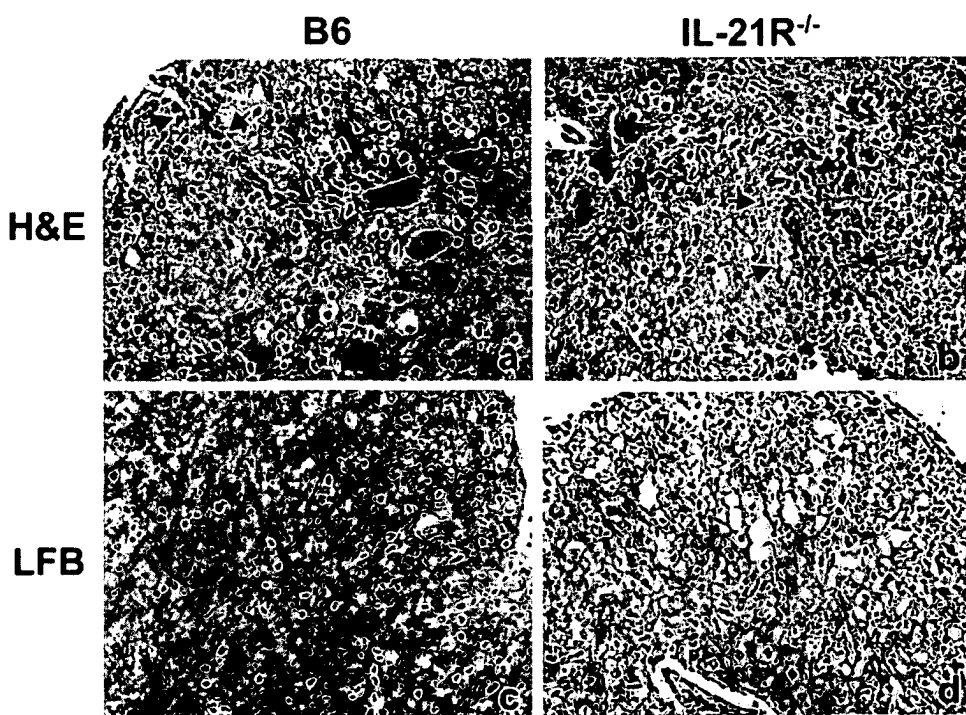


Fig. 10B



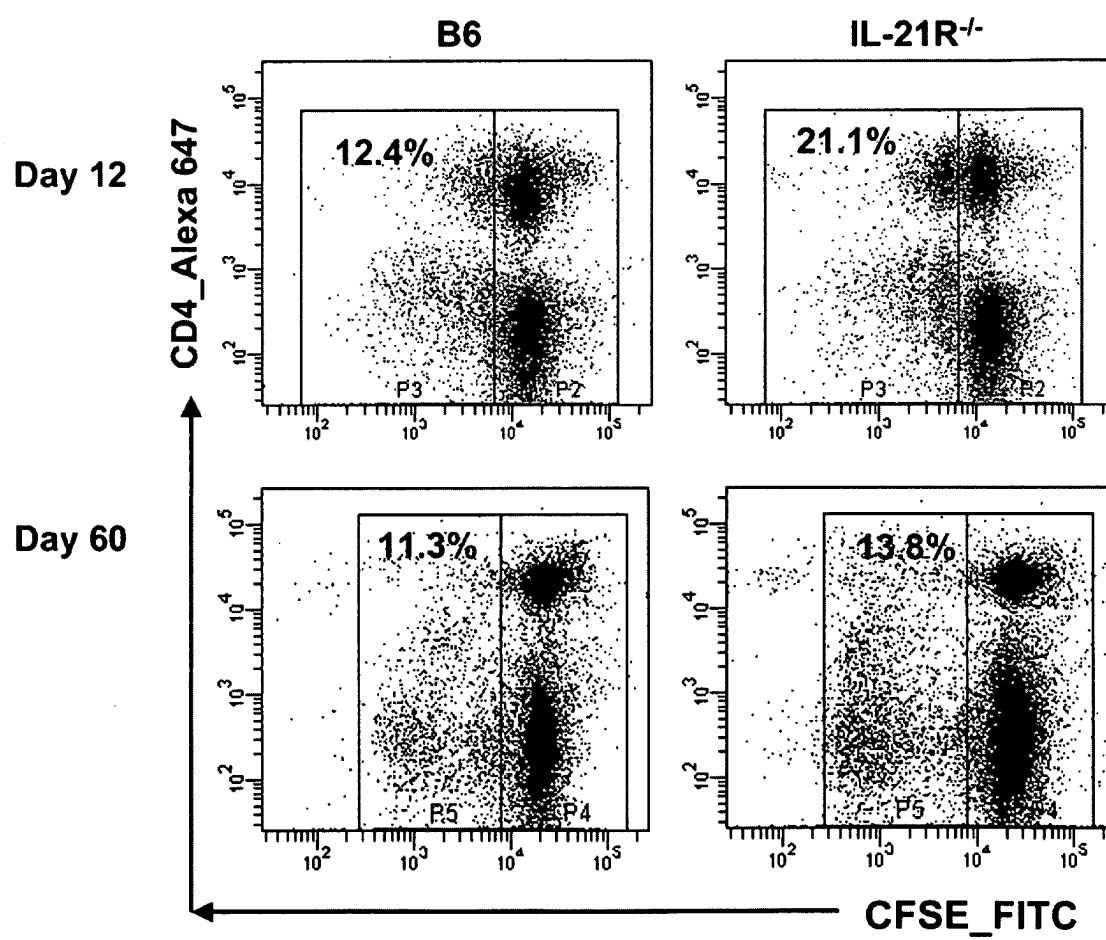
**Fig. 11**



Fig. 12A

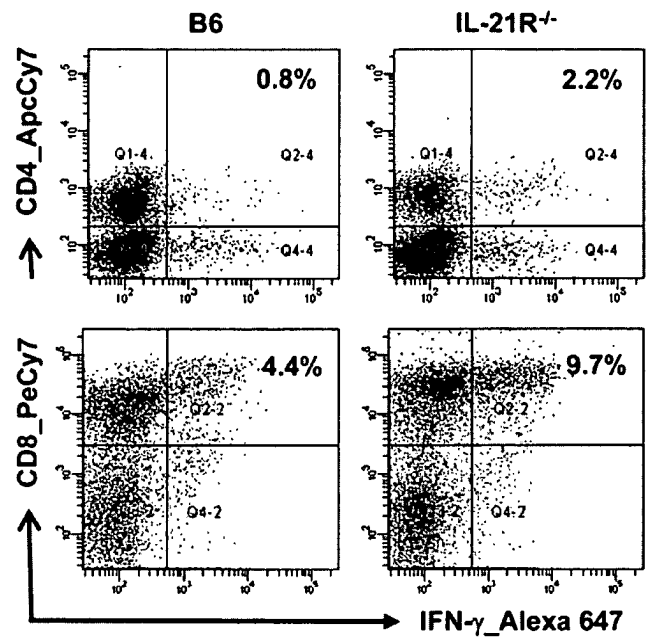


Fig. 12B

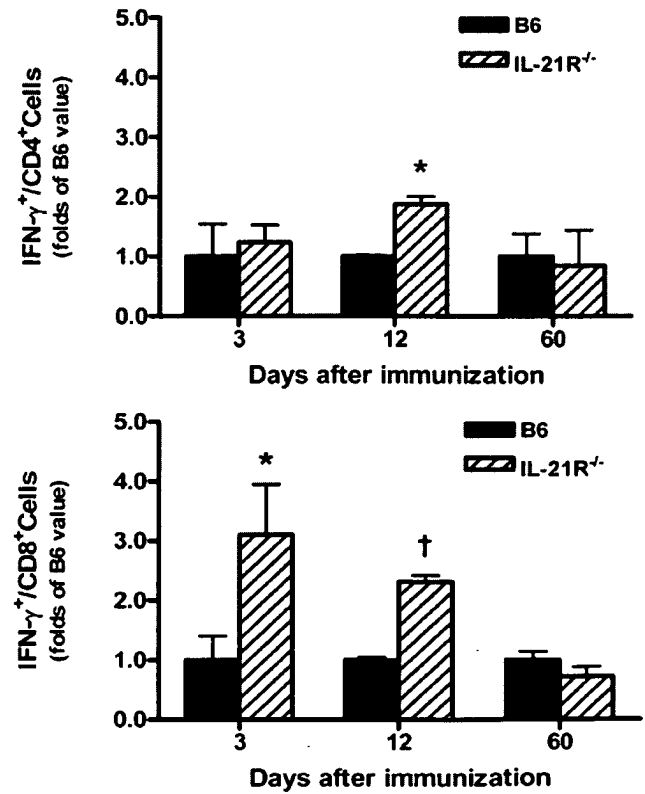


Fig. 12C

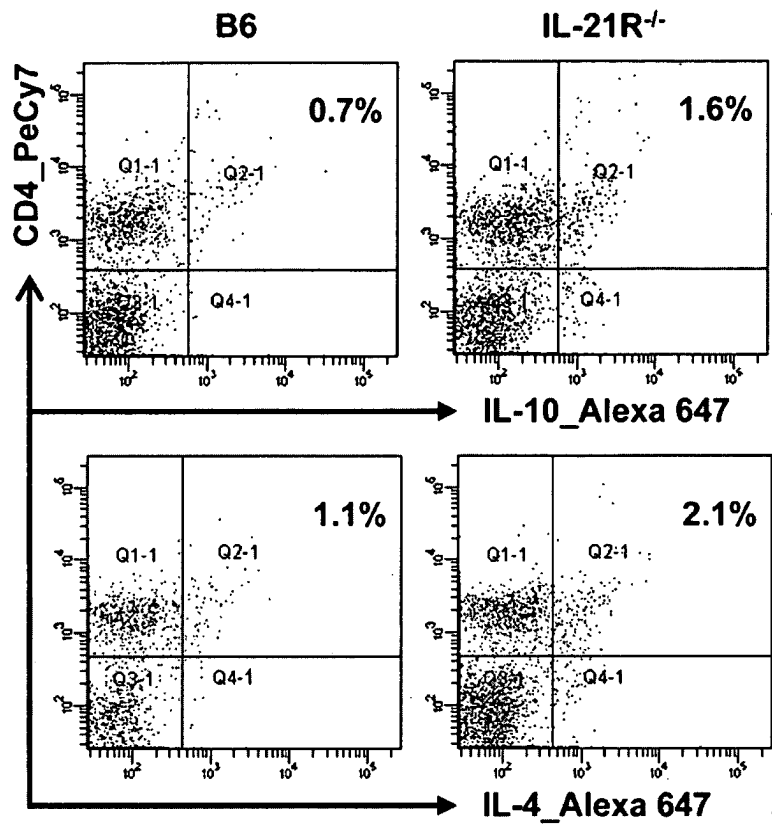


Fig. 12D

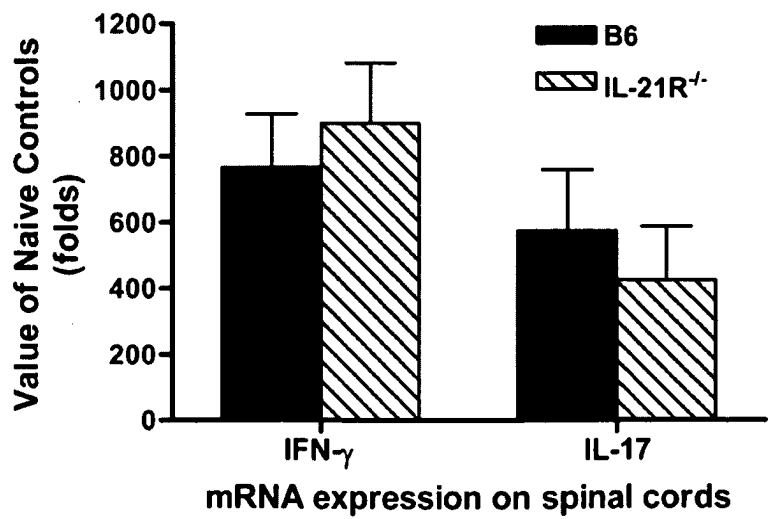


Fig. 13

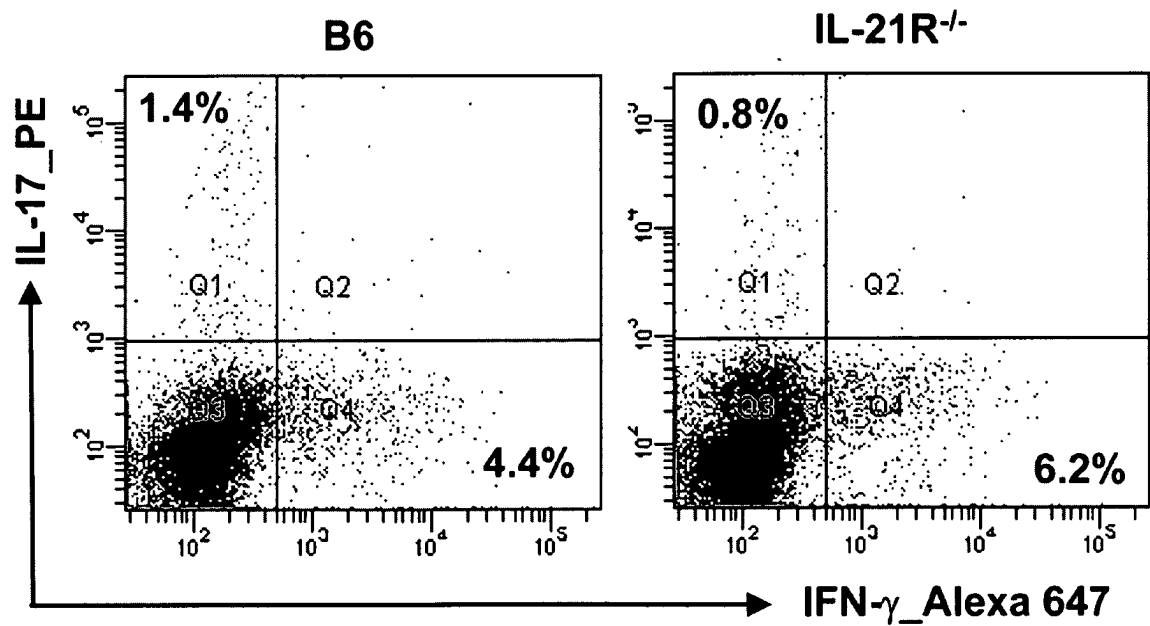


Fig. 14A

Treg cells

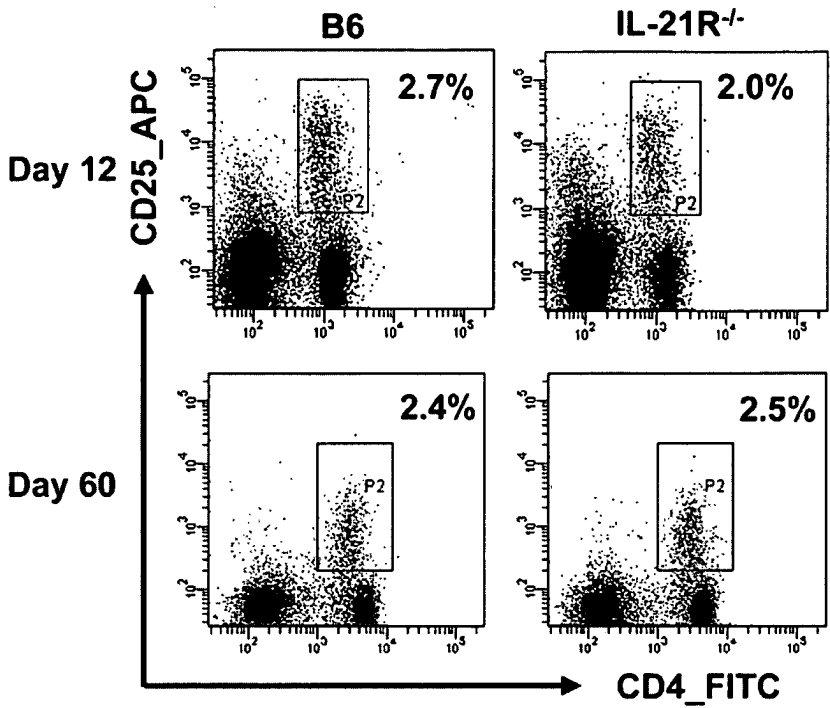


Fig. 14B

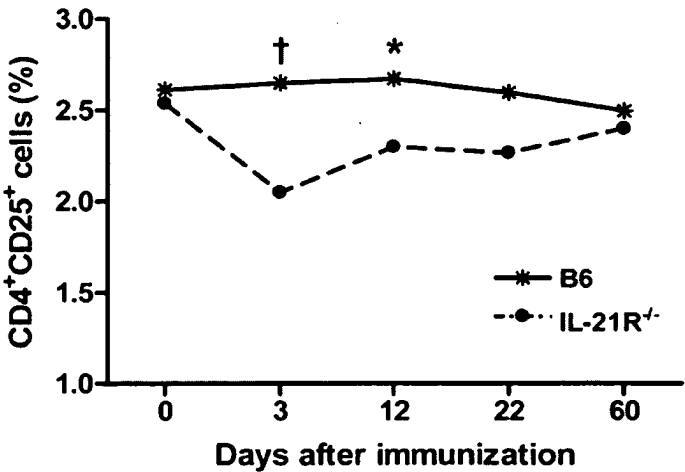


Fig. 14C

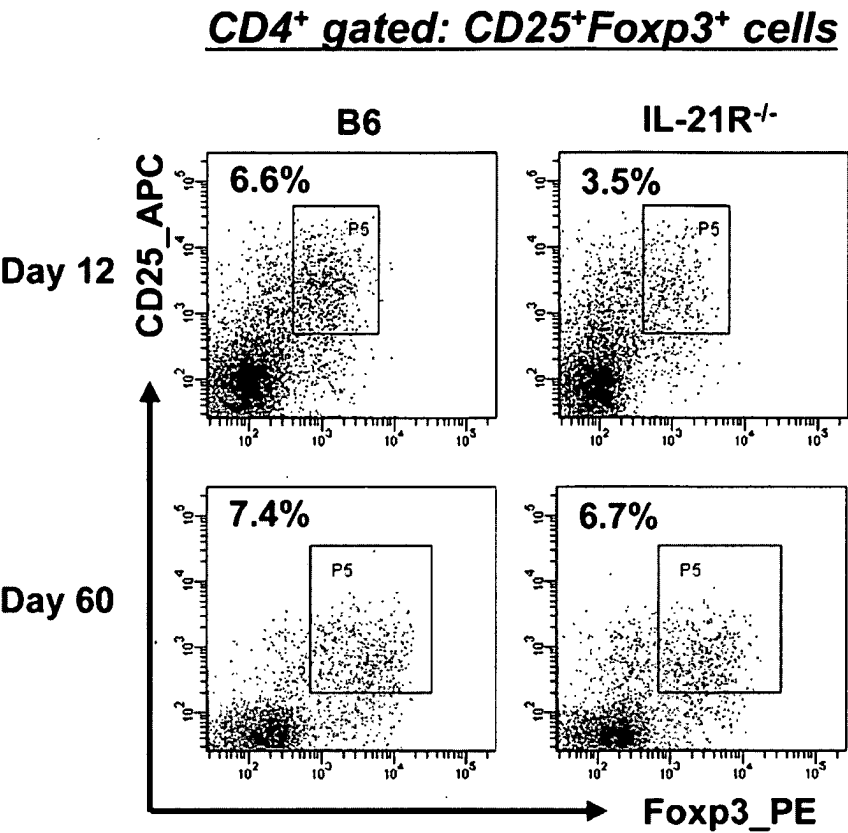
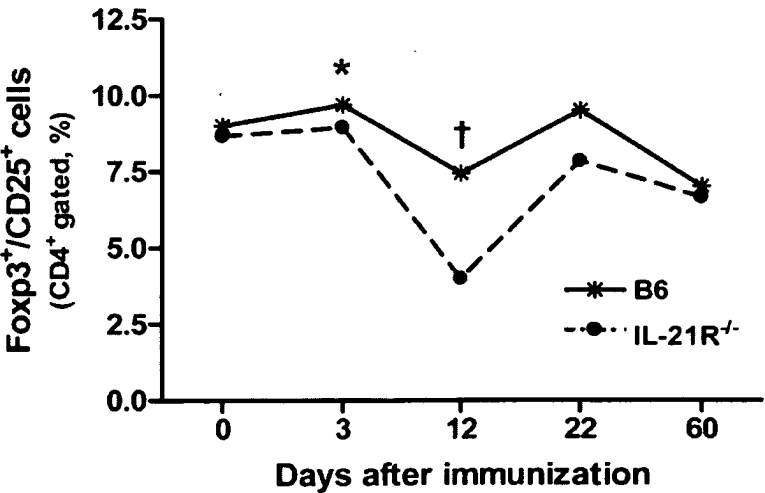


Fig. 14D



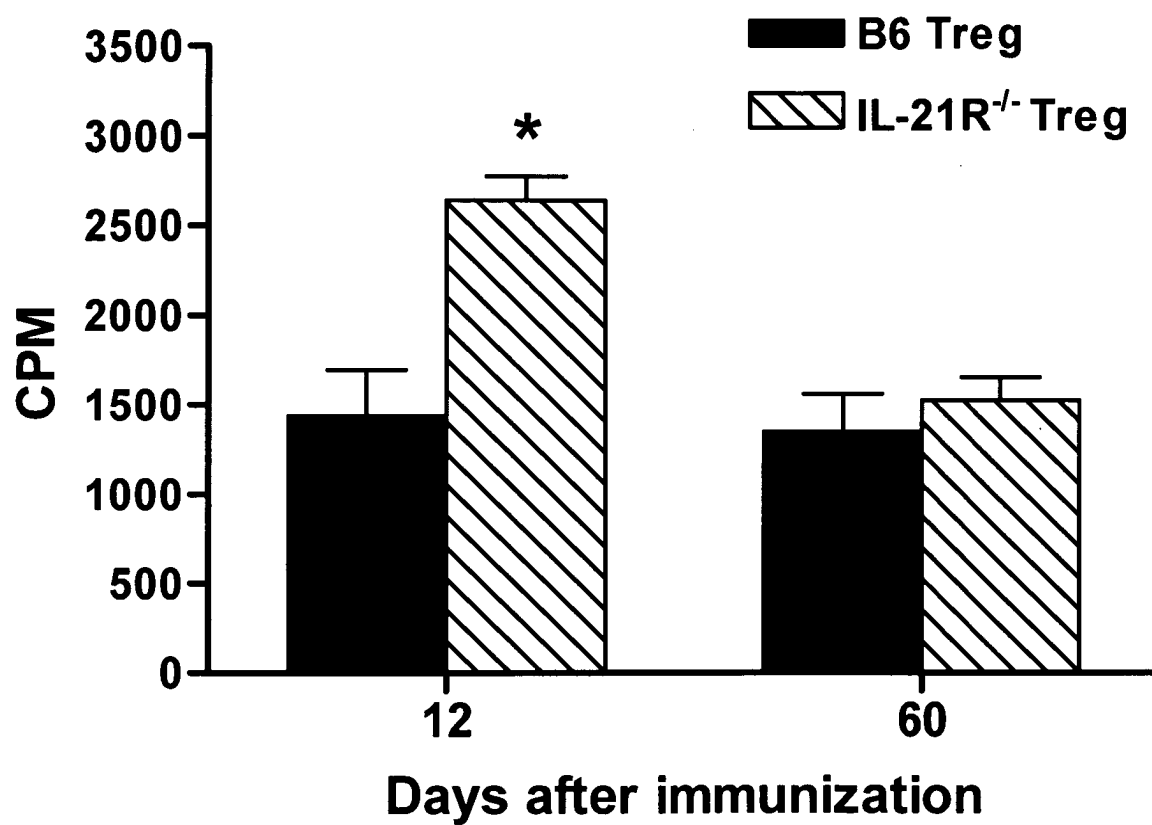
**Fig. 15**

Fig. 16A

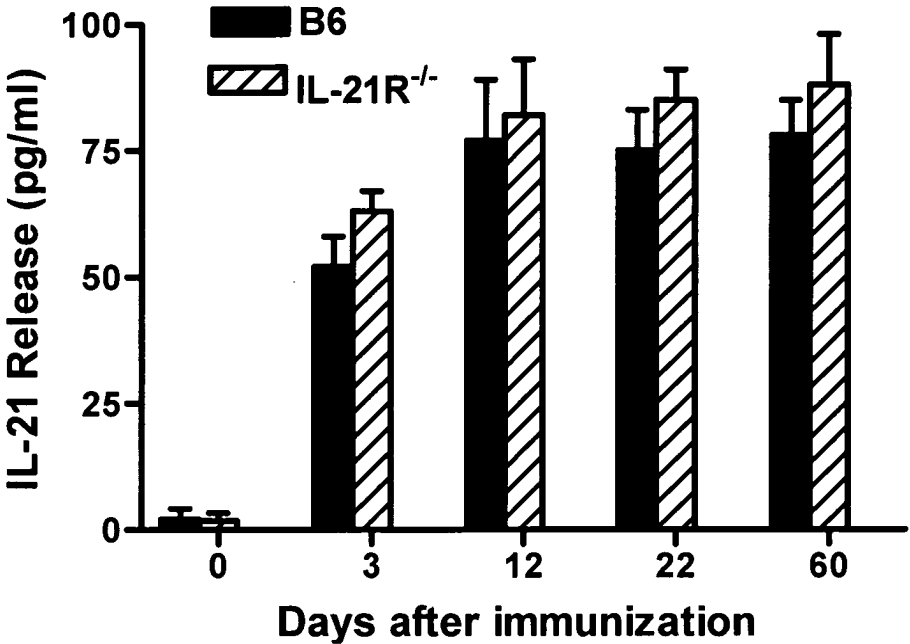


Fig. 16B

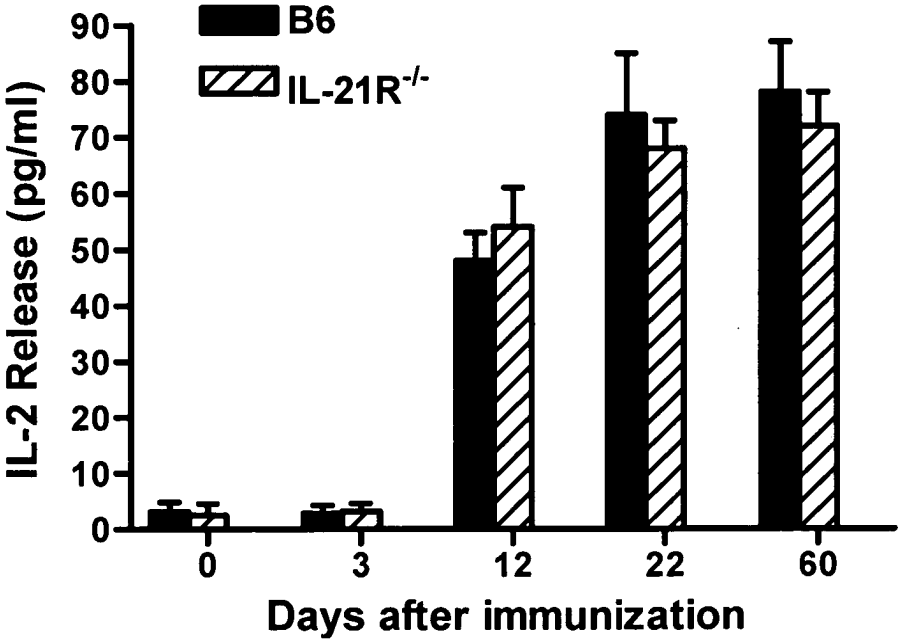


Fig. 16C

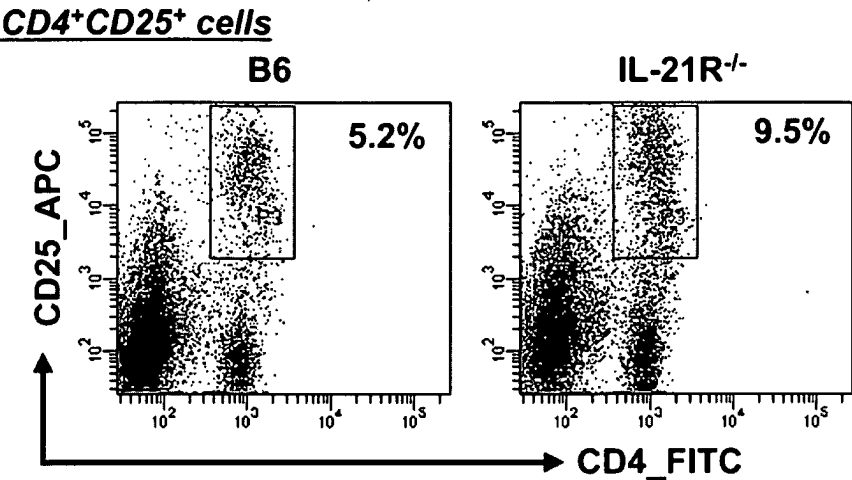


Fig. 16D

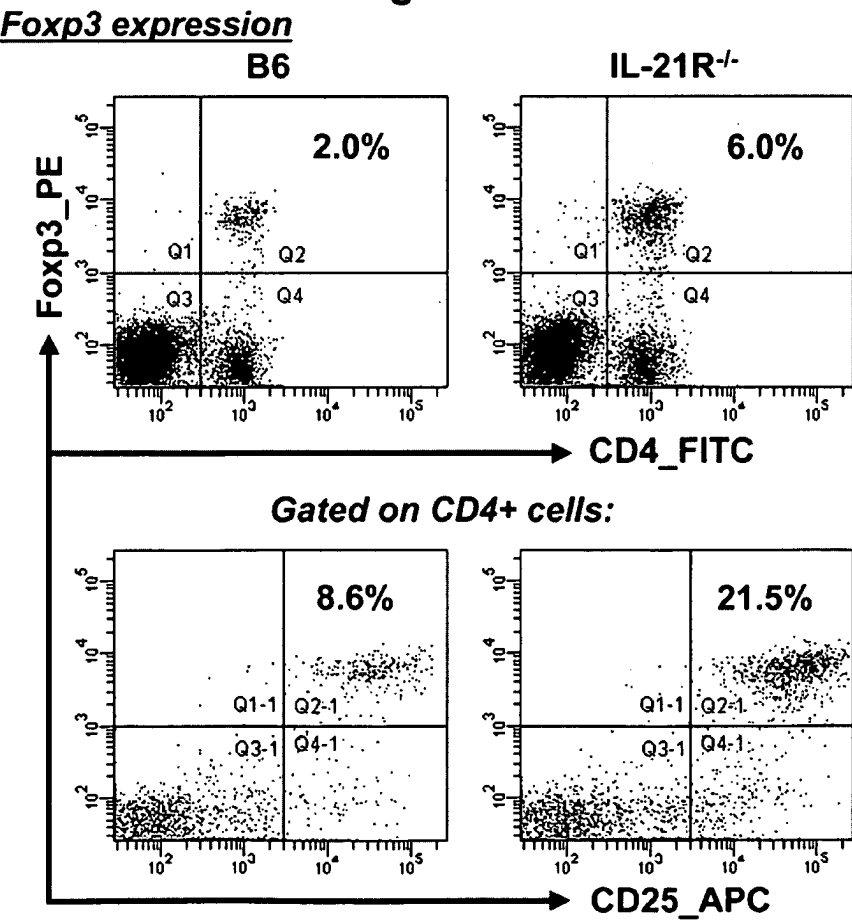




Fig. 17A

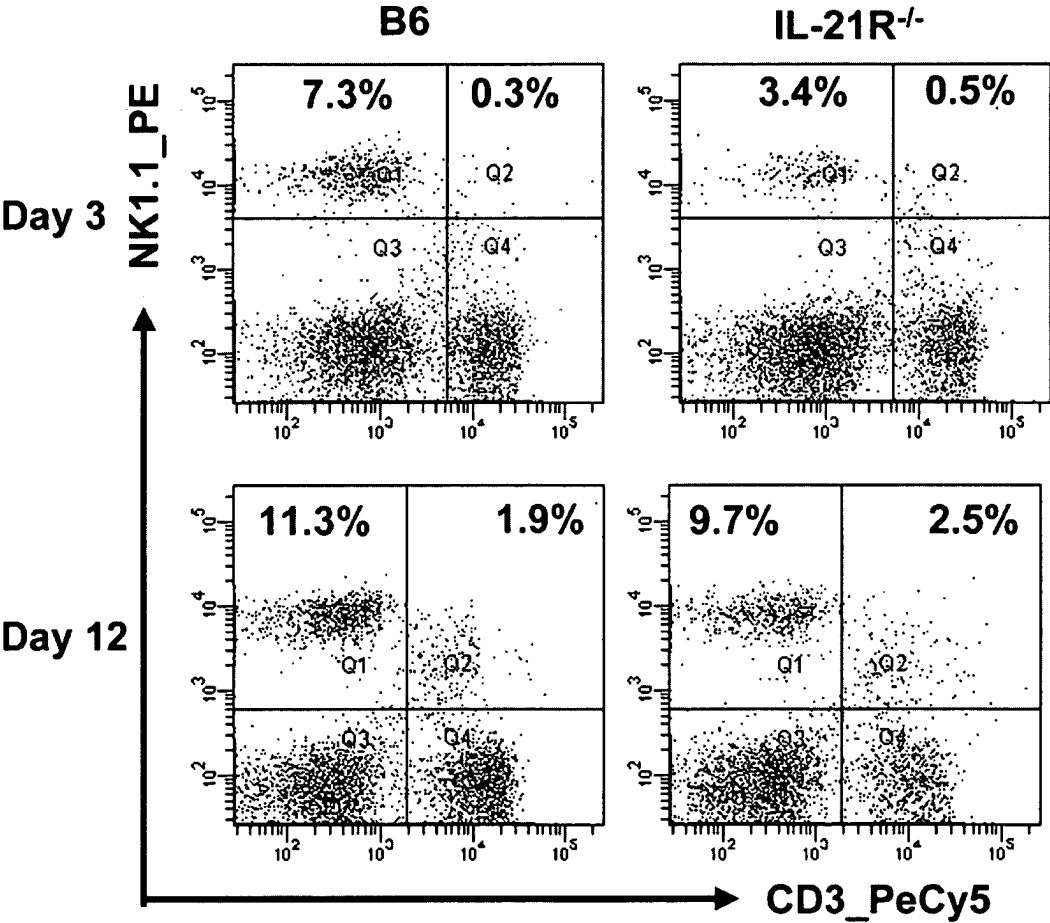


Fig. 17B

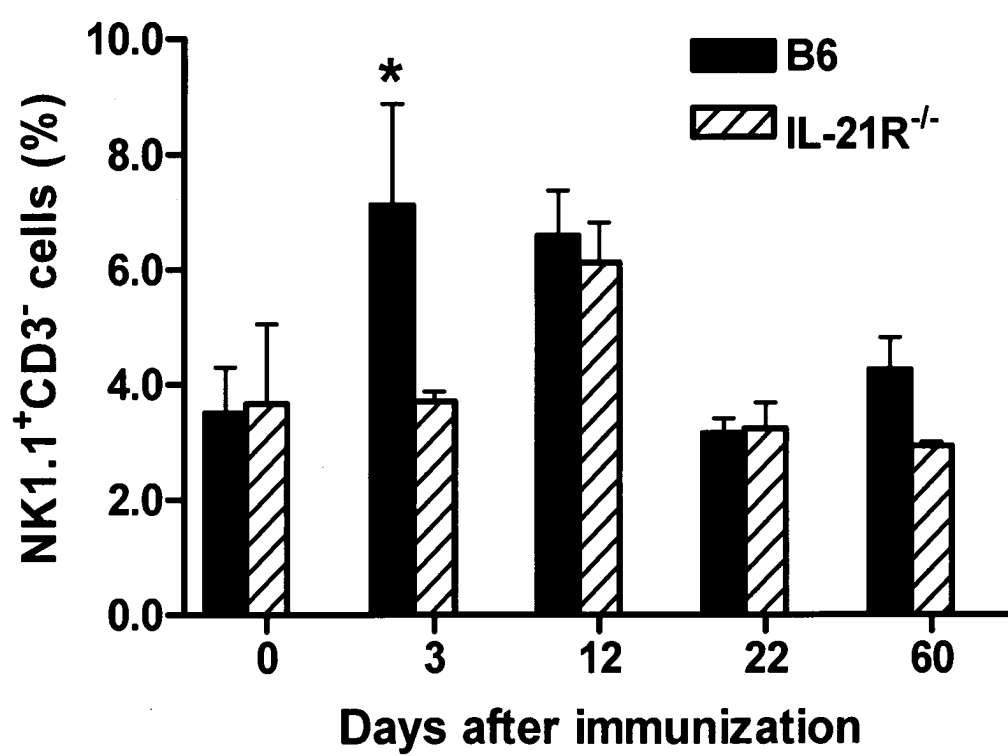


Fig. 17C

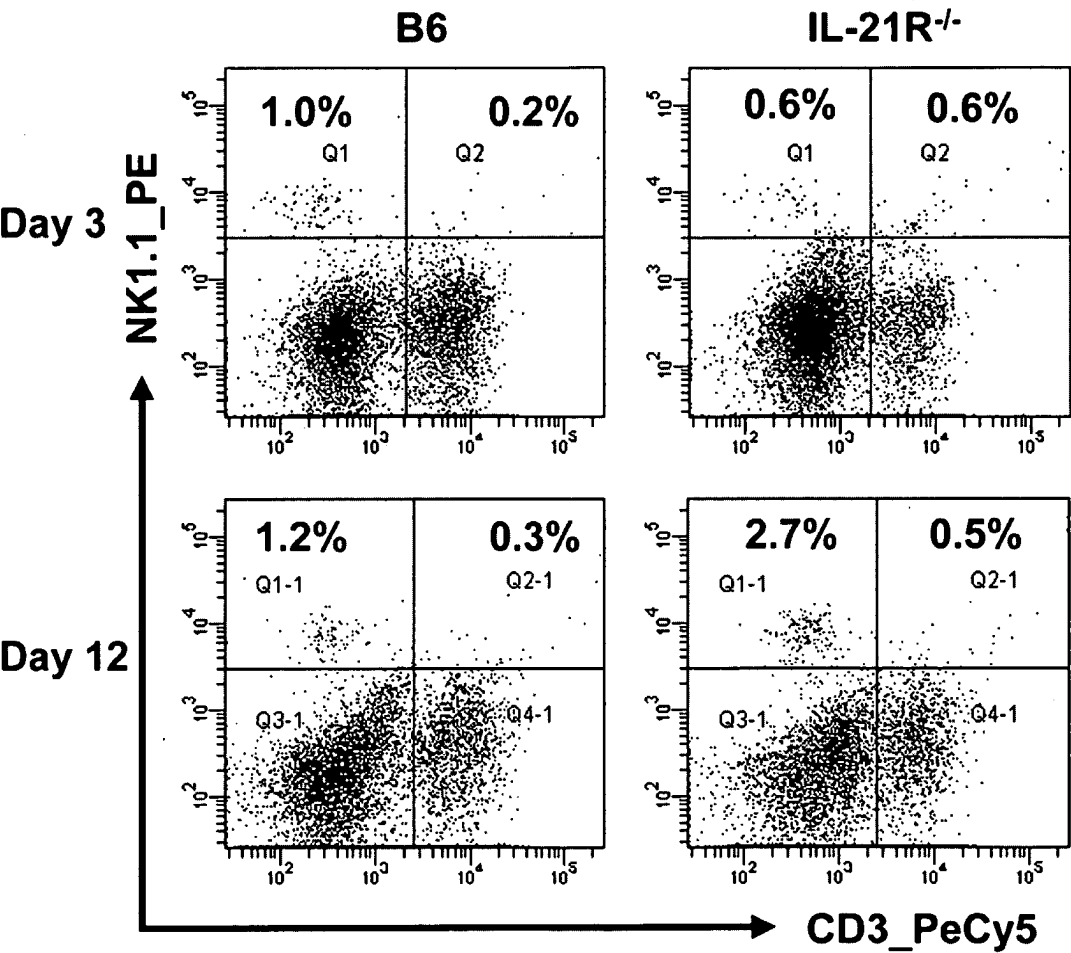
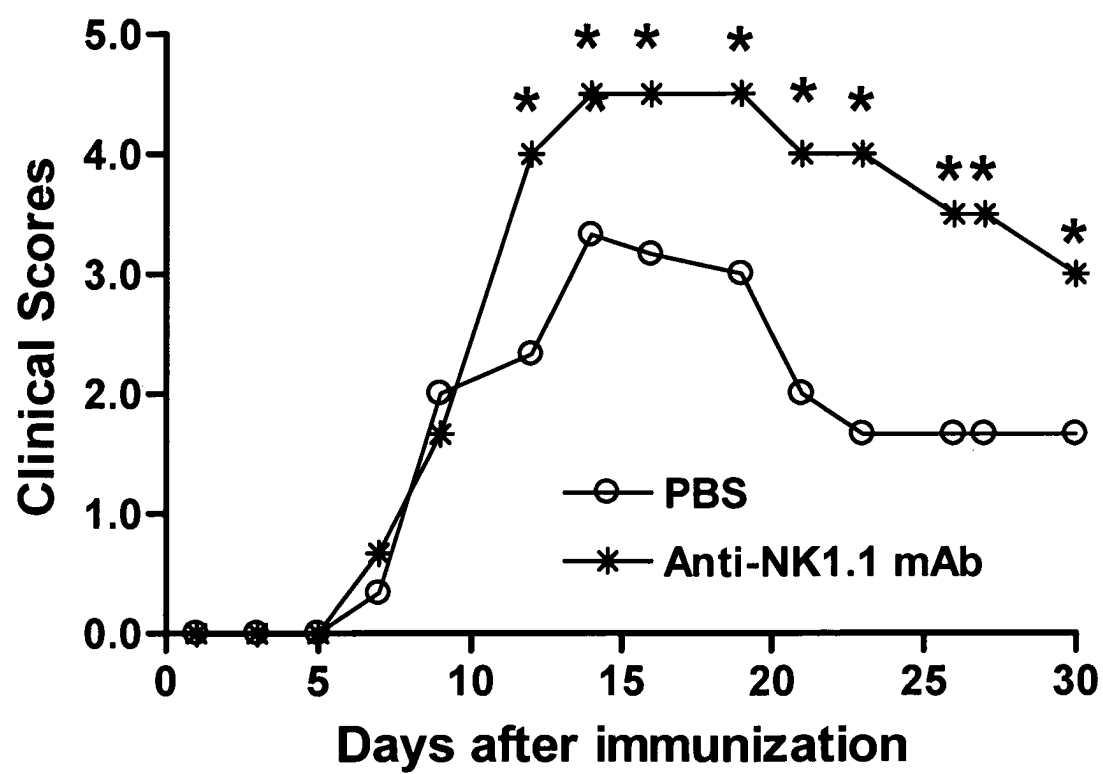


Fig. 17D



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Gly Trp Gly Cys Pro Asp Leu Val Cys Tyr Thr Asp Tyr Leu Gln Thr  
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Val Ile Cys Ile Leu Glu Met Trp Asn Leu His Pro Ser Thr Leu Thr  
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Cys Ser Leu His Arg Ser Ala His Asn Ala Thr His Ala Thr Tyr Thr  
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Cys His Met Asp Val Phe His Phe Met Ala Asp Asp Ile Phe Ser Val  
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## 01997069600PCST25.txt

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Leu	Leu	Ala 115	Glu	Ser	Ile	Lys	Pro 120	Ala	Pro	Pro	Phe	Asn 125	Val	Thr	Val	
acc	ttc	tca	gga	cag	tat	aat	atc	tcc	tgg	cgc	tca	gat	tac	gaa	gac	552
Thr	Phe 130	Ser	Gly	Gln	Tyr	Asn 135	Ile	Ser	Trp	Arg	Ser 140	Asp	Tyr	Glu	Asp	
cct	gcc	ttc	tac	atg	ctg	aag	ggc	aag	ctt	cag	tat	gag	ctg	cag	tac	600
Pro 145	Ala	Phe	Tyr	Met	Leu 150	Lys	Gly	Lys	Leu	Gln 155	Tyr	Glu	Leu	Gln	Tyr 160	
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Arg	Asn	Arg	Gly	Asp 165	Pro	Trp	Ala	Val	Ser 170	Pro	Arg	Arg	Lys	Leu 175	Ile	
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Ser	Val	Asp	Ser 180	Arg	Ser	Val	Ser	Leu 185	Leu	Pro	Leu	Glu	Phe 190	Arg	Lys	
gac	tcg	agc	tat	gag	ctg	cag	gtg	cgg	gca	ggg	ccc	atg	cct	ggc	tcc	744
Asp	Ser	Ser 195	Tyr	Glu	Leu	Gln	Val 200	Arg	Ala	Gly	Pro	Met 205	Pro	Gly	Ser	
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Ser	Tyr 210	Gln	Gly	Thr	Trp	Ser 215	Glu	Trp	Ser	Asp	Pro 220	Val	Ile	Phe	Gln	
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Thr 225	Gln	Ser	Glu	Glu	Leu 230	Lys	Glu	Gly	Trp	Asn 235	Pro	His	Leu	Leu	Leu 240	
ctc	ctc	ctg	ctt	gtc	ata	gtc	ttc	att	cct	gcc	ttc	tgg	agc	ctg	aag	888
Leu	Leu	Leu	Leu	Val 245	Ile	Val	Phe	Ile	Pro 250	Ala	Phe	Trp	Ser	Leu 255	Lys	
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Thr	His	Pro	Leu 260	Trp	Arg	Leu	Trp	Lys 265	Lys	Ile	Trp	Ala	Val 270	Pro	Ser	
cct	gag	cgg	ttc	ttc	atg	ccc	ctg	tac	aag	ggc	tgc	agc	gga	gac	ttc	984
Pro	Glu	Arg 275	Phe	Phe	Met	Pro	Leu 280	Tyr	Lys	Gly	Cys	Ser 285	Gly	Asp	Phe	
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Lys	Lys 290	Trp	Val	Gly	Ala	Pro 295	Phe	Thr	Gly	Ser	Ser 300	Leu	Glu	Leu	Gly	
ccc	tgg	agc	cca	gag	gtg	ccc	tcc	acc	ctg	gag	gtg	tac	agc	tgc	cac	1080
Pro 305	Trp	Ser	Pro	Glu	Val 310	Pro	Ser	Thr	Leu	Glu 315	Val	Tyr	Ser	Cys	His 320	
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Pro	Pro	Arg	Ser	Pro 325	Ala	Lys	Arg	Leu	Gln 330	Leu	Thr	Glu	Leu	Gln 335	Glu	
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Pro	Ala	Glu	Leu 340	Val	Glu	Ser	Asp	Gly 345	Val	Pro	Lys	Pro	Ser 350	Phe	Trp	

## 01997069600PCST25.txt

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cgg cca tac ggc ctg gtg tcc att gac aca gtg act gtg cta gat gca Arg Pro Tyr Gly Leu Val Ser Ile Asp Thr Val Thr Val Leu Asp Ala 370 375 380	1272
gag ggg cca tgc acc tgg ccc tgc agc tgt gag gat gac ggc tac cca Glu Gly Pro Cys Thr Trp Pro Cys Ser Cys Glu Asp Asp Gly Tyr Pro 385 390 395	1320
gcc ctg gac ctg gat gct ggc ctg gag ccc agc cca ggc cta gag gac Ala Leu Asp Leu Asp Ala Gly Leu Glu Pro Ser Pro Gly Leu Glu Asp 405 410 415	1368
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Leu Thr Trp Gln Asp Gln Tyr Glu Glu Leu Lys Asp Glu Ala Thr Ser  
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Cys Ser Leu His Arg Ser Ala His Asn Ala Thr His Ala Thr Tyr Thr  
 65 70 75 80



Cys His Met Asp Val Phe His Phe Met Ala Asp Asp Ile Phe Ser Val  
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Asn Ile Thr Asp Gln Ser Gly Asn Tyr Ser Gln Glu Cys Gly Ser Phe  
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Leu Leu Ala Glu Ser Ile Lys Pro Ala Pro Pro Phe Asn Val Thr Val  
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Pro Ala Phe Tyr Met Leu Lys Gly Lys Leu Gln Tyr Glu Leu Gln Tyr  
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Arg Asn Arg Gly Asp Pro Trp Ala Val Ser Pro Arg Arg Lys Leu Ile  
165 170 175

Ser Val Asp Ser Arg Ser Val Ser Leu Leu Pro Leu Glu Phe Arg Lys  
180 185 190

Asp Ser Ser Tyr Glu Leu Gln Val Arg Ala Gly Pro Met Pro Gly Ser  
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Ser Tyr Gln Gly Thr Trp Ser Glu Trp Ser Asp Pro Val Ile Phe Gln  
210 215 220

Thr Gln Ser Glu Glu Leu Lys Glu Gly Trp Asn Pro His Leu Leu Leu  
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Thr His Pro Leu Trp Arg Leu Trp Lys Lys Ile Trp Ala Val Pro Ser  
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Pro Glu Arg Phe Phe Met Pro Leu Tyr Lys Gly Cys Ser Gly Asp Phe  
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Lys Lys Trp Val Gly Ala Pro Phe Thr Gly Ser Ser Leu Glu Leu Gly  
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01997069600PCST25.txt

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355 360 365

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370 375 380

Glu Gly Pro Cys Thr Trp Pro Cys Ser Cys Glu Asp Asp Gly Tyr Pro  
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Ala Leu Asp Leu Asp Ala Gly Leu Glu Pro Ser Pro Gly Leu Glu Asp  
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Ala Gly Ser Pro Gly Leu Gly Gly Pro Leu Gly Ser Leu Leu Asp Arg  
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Leu Lys Pro Pro Leu Ala Asp Gly Glu Asp Trp Ala Gly Gly Leu Pro  
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Trp Gly Gly Arg Ser Pro Gly Gly Val Ser Glu Ser Glu Ala Gly Ser  
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## 01997069600PCST25.txt

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Cys Tyr Thr Asp Tyr Leu Trp Thr Ile Thr Cys Val Leu Glu Thr Arg						
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Ser Pro Asn Pro Ser Ile Leu Ser Leu Thr Trp Gln Asp Glu Tyr Glu						
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Glu Leu Gln Asp Gln Glu Thr Phe Cys Ser Leu His Arg Ser Gly His						
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Asn Thr Thr His Ile Trp Tyr Thr Cys His Met Arg Leu Ser Gln Phe						
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Leu Ser Asp Glu Val Phe Ile Val Asn Val Thr Asp Gln Ser Gly Asn						
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Lys Leu Gln Tyr Glu Leu Gln Tyr Arg Asn Leu Arg Asp Pro Tyr Ala						
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Val Arg Pro Val Thr Lys Leu Ile Ser Val Asp Ser Arg Asn Val Ser						
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Leu Leu Pro Glu Glu Phe His Lys Asp Ser Ser Tyr Gln Leu Gln Val						

## 01997069600PCST25.txt

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Trp	Ser	Asp	Pro	Val	Ile	Phe	Gln	Thr	Gln	Ala	Gly	Glu	Pro	Glu	Ala								
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Gly	Trp	Asp	Pro	His	Met	Leu	Leu	Leu	Leu	Ala	Val	Leu	Ile	Ile	Val								
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Ser	Cys	Gly	Cys	Val	Ser	Gly	Ser	Gly	Leu	Arg	Leu	Gly	Gly	Ser	Pro								
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## 01997069600PCST25.txt

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Leu Thr Trp Gln Asp Glu Tyr Glu Glu Leu Gln Asp Gln Glu Thr Phe  
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Cys Ser Leu His Arg Ser Gly His Asn Thr Thr His Ile Trp Tyr Thr  
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Cys His Met Arg Leu Ser Gln Phe Leu Ser Asp Glu Val Phe Ile Val  
 85 90 95

Asn Val Thr Asp Gln Ser Gly Asn Asn Ser Gln Glu Cys Gly Ser Phe  
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Val Leu Ala Glu Ser Ile Lys Pro Ala Pro Pro Leu Asn Val Thr Val  
 115 120 125

Ala Phe Ser Gly Arg Tyr Asp Ile Ser Trp Asp Ser Ala Tyr Asp Glu  
 130 135 140

Pro Ser Asn Tyr Val Leu Arg Gly Lys Leu Gln Tyr Glu Leu Gln Tyr  
 145 150 155 160

Arg Asn Leu Arg Asp Pro Tyr Ala Val Arg Pro Val Thr Lys Leu Ile  
 165 170 175

Ser Val Asp Ser Arg Asn Val Ser Leu Leu Pro Glu Glu Phe His Lys  
 180 185 190

Asp Ser Ser Tyr Gln Leu Gln Val Arg Ala Ala Pro Gln Pro Gly Thr  
 195 200 205

Ser Phe Arg Gly Thr Trp Ser Glu Trp Ser Asp Pro Val Ile Phe Gln  
 210 215 220

Thr Gln Ala Gly Glu Pro Glu Ala Gly Trp Asp Pro His Met Leu Leu  
 225 230 235 240

Leu Leu Ala Val Leu Ile Ile Val Leu Val Phe Met Gly Leu Lys Ile  
 245 250 255

His Leu Pro Trp Arg Leu Trp Lys Lys Ile Trp Ala Pro Val Pro Thr  
 260 265 270

Pro Glu Ser Phe Phe Gln Pro Leu Tyr Arg Glu His Ser Gly Asn Phe  
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Lys Lys Trp Val Asn Thr Pro Phe Thr Ala Ser Ser Ile Glu Leu Val  
 290 295 300

01997069600PCST25.txt

Pro Gln Ser Ser Thr Thr Thr Ser Ala Leu His Leu Ser Leu Tyr Pro  
305 310 315 320

Ala Lys Glu Lys Lys Phe Pro Gly Leu Pro Gly Leu Glu Glu Gln Leu  
325 330 335

Glu Cys Asp Gly Met Ser Glu Pro Gly His Trp Cys Ile Ile Pro Leu  
340 345 350

Ala Ala Gly Gln Ala Val Ser Ala Tyr Ser Glu Glu Arg Asp Arg Pro  
355 360 365

Tyr Gly Leu Val Ser Ile Asp Thr Val Thr Val Gly Asp Ala Glu Gly  
370 375 380

Leu Cys Val Trp Pro Cys Ser Cys Glu Asp Asp Gly Tyr Pro Ala Met  
385 390 395 400

Asn Leu Asp Ala Gly Arg Glu Ser Gly Pro Asn Ser Glu Asp Leu Leu  
405 410 415

Leu Val Thr Asp Pro Ala Phe Leu Ser Cys Gly Cys Val Ser Gly Ser  
420 425 430

Gly Leu Arg Leu Gly Gly Ser Pro Gly Ser Leu Leu Asp Arg Leu Arg  
435 440 445

Leu Ser Phe Ala Lys Glu Gly Asp Trp Thr Ala Asp Pro Thr Trp Arg  
450 455 460

Thr Gly Ser Pro Gly Gly Gly Ser Glu Ser Glu Ala Gly Ser Pro Pro  
465 470 475 480

Gly Leu Asp Met Asp Thr Phe Asp Ser Gly Phe Ala Gly Ser Asp Cys  
485 490 495

Gly Ser Pro Val Glu Thr Asp Glu Gly Pro Pro Arg Ser Tyr Leu Arg  
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Gln Trp Val Val Arg Thr Pro Pro Val Asp Ser Gly Ala Gln Ser  
515 520 525

Ser

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<212> PRT  
 <213> homo sapiens

<400> 5

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Gln Phe Thr Cys Phe Tyr Asn Ser Arg Ala Asn Ile Ser Cys Val Trp  
 35 40 45

Ser Gln Asp Gly Ala Leu Gln Asp Thr Ser Cys Gln Val His Ala Trp  
 50 55 60

Pro Asp Arg Arg Arg Trp Asn Gln Thr Cys Glu Leu Leu Pro Val Ser  
 65 70 75 80

Gln Ala Ser Trp Ala Cys Asn Leu Ile Leu Gly Ala Pro Asp Ser Gln  
 85 90 95

Lys Leu Thr Thr Val Asp Ile Val Thr Leu Arg Val Leu Cys Arg Glu  
 100 105 110

Gly Val Arg Trp Arg Val Met Ala Ile Gln Asp Phe Lys Pro Phe Glu  
 115 120 125

Asn Leu Arg Leu Met Ala Pro Ile Ser Leu Gln Val Val His Val Glu  
 130 135 140

Thr His Arg Cys Asn Ile Ser Trp Glu Ile Ser Gln Ala Ser His Tyr  
 145 150 155 160

Phe Glu Arg His Leu Glu Phe Glu Ala Arg Thr Leu Ser Pro Gly His  
 165 170 175

Thr Trp Glu Glu Ala Pro Leu Leu Thr Leu Lys Gln Lys Gln Glu Trp  
 180 185 190

Ile Cys Leu Glu Thr Leu Thr Pro Asp Thr Gln Tyr Glu Phe Gln Val  
 195 200 205

Arg Val Lys Pro Leu Gln Gly Glu Phe Thr Thr Trp Ser Pro Trp Ser  
 210 215 220

Gln Pro Leu Ala Phe Arg Thr Lys Pro Ala Ala Leu Gly Lys Asp Thr  
 225 230 235 240



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Ile Pro Trp Leu Gly His Leu Leu Val Gly Leu Ser Gly Ala Phe Gly  
245 250 255

Phe Ile Ile Leu Val Tyr Leu Leu Ile Asn Cys Arg Asn Thr Gly Pro  
260 265 270

Trp Leu Lys Lys Val Leu Lys Cys Asn Thr Pro Asp Pro Ser Lys Phe  
275 280 285

Phe Ser Gln Leu Ser Ser Glu His Gly Gly Asp Val Gln Lys Trp Leu  
290 295 300

Ser Ser Pro Phe Pro Ser Ser Ser Phe Ser Pro Gly Gly Leu Ala Pro  
305 310 315 320

Glu Ile Ser Pro Leu Glu Val Leu Glu Arg Asp Lys Val Thr Gln Leu  
325 330 335

Leu Leu Gln Gln Asp Lys Val Pro Glu Pro Ala Ser Leu Ser Ser Asn  
340 345 350

His Ser Leu Thr Ser Cys Phe Thr Asn Gln Gly Tyr Phe Phe Phe His  
355 360 365

Leu Pro Asp Ala Leu Glu Ile Glu Ala Cys Gln Val Tyr Phe Thr Tyr  
370 375 380

Asp Pro Tyr Ser Glu Glu Asp Pro Asp Glu Gly Val Ala Gly Ala Pro  
385 390 395 400

Thr Gly Ser Ser Pro Gln Pro Leu Gln Pro Leu Ser Gly Glu Asp Asp  
405 410 415

Ala Tyr Cys Thr Phe Pro Ser Arg Asp Asp Leu Leu Leu Phe Ser Pro  
420 425 430

Ser Leu Leu Gly Gly Pro Ser Pro Pro Ser Thr Ala Pro Gly Gly Ser  
435 440 445

Gly Ala Gly Glu Glu Arg Met Pro Pro Ser Leu Gln Glu Arg Val Pro  
450 455 460

Arg Asp Trp Asp Pro Gln Pro Leu Gly Pro Pro Thr Pro Gly Val Pro  
465 470 475 480

Asp Leu Val Asp Phe Gln Pro Pro Pro Glu Leu Val Leu Arg Glu Ala

485

490

495

Gly Glu Glu Val Pro Asp Ala Gly Pro Arg Glu Gly Val Ser Phe Pro  
 500 505 510

Trp Ser Arg Pro Pro Gly Gln Gly Glu Phe Arg Ala Leu Asn Ala Arg  
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Gln Asp Pro Thr His Leu Val  
 545 550

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 <211> 224  
 <212> PRT  
 <213> homo sapiens

<400> 7

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Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro  
 35 40 45

Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala  
 50 55 60

Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val  
 65 70 75 80

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr  
 85 90 95

Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Val Pro Ile Glu Lys Thr  
 100 105 110

01997069600PCST25.txt

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu  
115 120 125

Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys  
130 135 140

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser  
145 150 155 160

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp  
165 170 175

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser  
180 185 190

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala  
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Gly Asn Met Glu Arg Ile Val Ile Cys Leu Met Val Ile Phe Leu Gly  
10 15 20

aca ctg gtc cac aaa tca agc tcc caa ggt caa gat cgc cac atg att 151  
Thr Leu Val His Lys Ser Ser Ser Gln Gly Gln Asp Arg His Met Ile  
25 30 35

aga atg cgt caa ctt ata gat att gtt gat cag ctg aaa aat tat gtg 199  
Arg Met Arg Gln Leu Ile Asp Ile Val Asp Gln Leu Lys Asn Tyr Val  
40 45 50

aat gac ttg gtc cct gaa ttt ctg cca gct cca gaa gat gta gag aca 247  
Asn Asp Leu Val Pro Glu Phe Leu Pro Ala Pro Glu Asp Val Glu Thr  
55 60 65

aac tgt gag tgg tca gct ttt tcc tgc ttt cag aag gcc caa cta aag 295  
Asn Cys Glu Trp Ser Ala Phe Ser Cys Phe Gln Lys Ala Gln Leu Lys  
Page 15

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Ser Ala Asn Thr Gly Asn Asn Glu Arg Ile Ile Asn Val Ser Ile Lys				90	95	100	
aag ctg aag agg aaa cca cct tcc aca aat gca ggg aga aga cag aaa	391						
Lys Leu Lys Arg Lys Pro Pro Ser Thr Asn Ala Gly Arg Arg Gln Lys				105	110	115	
cac aga cta aca tgc cct tca tgt gat tct tat gag aaa aaa cca ccc	439						
His Arg Leu Thr Cys Pro Ser Cys Asp Ser Tyr Glu Lys Lys Pro Pro				120	125	130	
aaa gaa ttc cta gaa aga ttc aaa tca ctt ctc caa aag atg att cat	487						
Lys Glu Phe Leu Glu Arg Phe Lys Ser Leu Leu Gln Lys Met Ile His				135	140	145	
cag cat ctg tcc tct aga aca cac gga agt gaa gat tcc tga	529						
Gln His Leu Ser Ser Arg Thr His Gly Ser Glu Asp Ser				150	155	160	
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ctttgtattc caagtggagg ag	611						

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 20 25 30

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 35 40 45

Leu Lys Asn Tyr Val Asn Asp Leu Val Pro Glu Phe Leu Pro Ala Pro  
 50 55 60

Glu Asp Val Glu Thr Asn Cys Glu Trp Ser Ala Phe Ser Cys Phe Gln  
 65 70 75 80

Lys Ala Gln Leu Lys Ser Ala Asn Thr Gly Asn Asn Glu Arg Ile Ile  
 85 90 95

Asn Val Ser Ile Lys Lys Leu Lys Arg Lys Pro Pro Ser Thr Asn Ala  
 100 105 110

Gly Arg Arg Gln Lys His Arg Leu Thr Cys Pro Ser Cys Asp Ser Tyr  
 Page 16

115

120

125

Glu Lys Lys Pro Pro Lys Glu Phe Leu Glu Arg Phe Lys Ser Leu Leu  
 130 135 140

Gln Lys Met Ile His Gln His Leu Ser Ser Arg Thr His Gly Ser Glu  
 145 150 155 160

Asp Ser

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 Glu Arg Thr Leu Val Cys Leu Val Val Ile Phe Leu Gly Thr Val Ala  
 5 10 15

cat aaa tca agc ccc caa ggg cca gat cgc ctc ctg att aga ctt cgt 152  
 His Lys Ser Ser Pro Gln Gly Pro Asp Arg Leu Leu Ile Arg Leu Arg  
 20 25 30

cac ctt att gac att gtt gaa cag ctg aaa atc tat gaa aat gac ttg 200  
 His Leu Ile Asp Ile Val Glu Gln Leu Lys Ile Tyr Glu Asn Asp Leu  
 35 40 45

gat cct gaa ctt cta tca gct cca caa gat gta aag ggg cac tgt gag 248  
 Asp Pro Glu Leu Leu Ser Ala Pro Gln Asp Val Lys Gly His Cys Glu  
 50 55 60 65

cat gca gct ttt gcc tgt ttt cag aag gcc aaa ctc aag cca tca aac 296  
 His Ala Ala Phe Ala Cys Phe Gln Lys Ala Lys Leu Lys Pro Ser Asn  
 70 75 80

cct gga aac aat aag aca ttc atc att gac ctc gtg gcc cag ctc agg 344  
 Pro Gly Asn Asn Lys Thr Phe Ile Ile Asp Leu Val Ala Gln Leu Arg  
 85 90 95

agg agg ctg cct gcc agg agg gga gga aag aaa cag aag cac ata gct 392  
 Arg Arg Leu Pro Ala Arg Arg Gly Gly Lys Lys Gln Lys His Ile Ala  
 100 105 110

aaa tgc cct tcc tgt gat tgc tat gag aaa agg aca ccc aaa gaa ttc 440  
 Lys Cys Pro Ser Cys Asp Ser Tyr Glu Lys Arg Thr Pro Lys Glu Phe  
 115 120 125

cta gaa aga cta aaa tgg ctc ctt caa aag atg att cat cag cat ctc 488

Leu Glu Arg Leu Lys Trp Leu Leu Gln Lys Met Ile His Gln His Leu  
 130 135 140 145

tcc tag aacacatagg acccgaagat tcctgaggat ccgagaagat tcccaggac 544  
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Arg His Leu Ile Asp Ile Val Glu Gln Leu Lys Ile Tyr Glu Asn Asp  
35 40 45

Leu Asp Pro Glu Leu Leu Ser Ala Pro Gln Asp Val Lys Gly His Cys  
50 55 60

Glu His Ala Ala Phe Ala Cys Phe Gln Lys Ala Lys Leu Lys Pro Ser  
65 70 75 80

Asn Pro Gly Asn Asn Lys Thr Phe Ile Ile Asp Leu Val Ala Gln Leu  
85 90 95

Arg Arg Arg Leu Pro Ala Arg Arg Gly Gly Lys Lys Gln Lys His Ile  
Page 19

100

105

110

Ala Lys Cys Pro Ser Cys Asp Ser Tyr Glu Lys Arg Thr Pro Lys Glu  
           115                          120                          125

Phe Leu Glu Arg Leu Lys Trp Leu Leu Gln Lys Met Ile His Gln His  
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Leu Ser  
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gcc cat aaa tca agc ccc caa agg cca gat cac ctt ctg att aga ctt	96
Ala His Lys Ser Pro Gln Arg Pro Asp His Leu Leu Ile Arg Leu	
20                          25                          30	
cgt cac ctt atg gac att gtt gaa cag ctg aaa atc tat gag aat gac	144
Arg His Leu Met Asp Ile Val Glu Gln Leu Lys Ile Tyr Glu Asn Asp	
35                          40                          45	
ttg gat cct gaa ctt cta aca gct cca caa gat gta aag ggg caa tgt	192
Leu Asp Pro Glu Leu Leu Thr Ala Pro Gln Asp Val Lys Gly Gln Cys	
50                          55                          60	
gag cac gaa gct ttt gcc tgt ttt cag aaa gcc aaa ctc aag cca tca	240
Glu His Glu Ala Phe Ala Cys Phe Gln Lys Ala Lys Leu Lys Pro Ser	
65                          70                          75                          80	
aac act gga aac aat aag aca ttc atc aac gac ttg ttg gca cag ctg	288
Asn Thr Gly Asn Asn Lys Thr Phe Ile Asn Asp Leu Leu Ala Gln Leu	
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agg agg agg ctg cct gct aag agg aca gga aat aaa cag agg cac atg	336
Arg Arg Arg Leu Pro Ala Lys Arg Thr Gly Asn Lys Gln Arg His Met	
100                          105                          110	
gct aaa tgc cct tcc tgt gat ttg tat gag aaa aaa aca ccc aaa gaa	384
Ala Lys Cys Pro Ser Cys Asp Leu Tyr Glu Lys Lys Thr Pro Lys Glu	
115                          120                          125	
ttc ctt gaa aga cta aaa tgg ctc ctt caa aag atg att cat cag cat	432
Phe Leu Glu Arg Leu Lys Trp Leu Leu Gln Lys Met Ile His Gln His	
130                          135                          140	
ctt tcc tag	441



Leu Ser  
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Arg His Leu Met Asp Ile Val Glu Gln Leu Lys Ile Tyr Glu Asn Asp  
35 40 45

Leu Asp Pro Glu Leu Leu Thr Ala Pro Gln Asp Val Lys Gly Gln Cys  
50 55 60

Glu His Glu Ala Phe Ala Cys Phe Gln Lys Ala Lys Leu Lys Pro Ser  
65 70 75 80

Asn Thr Gly Asn Asn Lys Thr Phe Ile Asn Asp Leu Leu Ala Gln Leu  
85 90 95

Arg Arg Arg Leu Pro Ala Lys Arg Thr Gly Asn Lys Gln Arg His Met  
100 105 110

Ala Lys Cys Pro Ser Cys Asp Leu Tyr Glu Lys Lys Thr Pro Lys Glu  
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Leu Ser  
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20 25 30

Ile Thr Cys Val Leu Glu Thr Arg Ser Pro Asn Pro Ser Ile Leu Ser  
35 40 45

Leu Thr Trp Gln Asp Glu Tyr Glu Glu Leu Gln Asp Gln Glu Thr Phe  
50 55 60

Cys Ser Leu His Arg Ser Gly His Asn Thr Thr His Ile Trp Tyr Thr  
65 70 75 80

Cys His Met Arg Leu Ser Gln Phe Leu Ser Asp Glu Val Phe Ile Val  
85 90 95

Asn Val Thr Asp Gln Ser Gly Asn Asn Ser Gln Glu Cys Gly Ser Phe  
100 105 110

Val Leu Ala Glu Ser Ile Lys Pro Ala Pro Pro Leu Asn Val Thr Val  
115 120 125

Ala Phe Ser Gly Arg Tyr Asp Ile Ser Trp Asp Ser Ala Tyr Asp Glu  
130 135 140

Pro Ser Asn Tyr Val Leu Arg Gly Lys Leu Gln Tyr Glu Leu Gln Tyr  
145 150 155 160

Arg Asn Leu Arg Asp Pro Tyr Ala Val Arg Pro Val Thr Lys Leu Ile  
165 170 175

Ser Val Asp Ser Arg Asn Val Ser Leu Leu Pro Glu Glu Phe His Lys  
180 185 190

Asp Ser Ser Tyr Gln Leu Gln Val Arg Ala Ala Pro Gln Pro Gly Thr  
195 200 205

Ser Phe Arg Gly Thr Trp Ser Glu Trp Ser Asp Pro Val Ile Phe Gln  
210 215 220

Thr Gln Ala Gly Glu Pro Glu Ala Gly Trp Asp Gly Ser Gly Ser Arg  
225 230 235 240

Glu Pro Arg Gly Pro Thr Ile Lys Pro Cys Pro Pro Cys Lys Cys Pro  
245 250 255

Ala Pro Asn Leu Glu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys

260

265

270

Ile Lys Asp Val Leu Met Ile Ser Leu Ser Pro Ile Val Thr Cys Val  
           275                          280                          285

Val Val Asp Val Ser Glu Asp Asp Pro Asp Val Gln Ile Ser Trp Phe  
       290                          295                          300

Val Asn Asn Val Glu Val His Thr Ala Gln Thr Gln Thr His Arg Glu  
   305                          310                          315                          320

Asp Tyr Asn Ser Thr Leu Arg Val Val Ser Ala Leu Pro Ile Gln His  
                           325                          330                          335

Gln Asp Trp Met Ser Gly Lys Glu Phe Lys Cys Lys Val Asn Asn Lys  
                           340                          345                          350

Asp Leu Pro Ala Pro Ile Glu Arg Thr Ile Ser Lys Pro Lys Gly Ser  
           355                          360                          365

Val Arg Ala Pro Gln Val Tyr Val Leu Pro Pro Pro Glu Glu Glu Met  
       370                          375                          380

Thr Lys Lys Gln Val Thr Leu Thr Cys Met Val Thr Asp Phe Met Pro  
   385                          390                          395                          400

Glu Asp Ile Tyr Val Glu Trp Thr Asn Asn Gly Lys Thr Glu Leu Asn  
                           405                          410                          415

Tyr Lys Asn Thr Glu Pro Val Leu Asp Ser Asp Gly Ser Tyr Phe Met  
                           420                          425                          430

Tyr Ser Lys Leu Arg Val Glu Lys Lys Asn Trp Val Glu Arg Asn Ser  
           435                          440                          445

Tyr Ser Cys Ser Val Val His Glu Gly Leu His Asn His His Thr Thr  
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Lys Ser Phe Ser Arg Thr Pro Gly Lys  
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 <213> homo sapiens

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<223> Xaa can be any naturally occurring amino acid

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Trp Ser Xaa Trp Ser  
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<213> artificial sequence

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His Ser Leu Gly Lys Trp Leu Gly His Pro Asp Lys Phe  
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Ser Gly Gly Gly Gly  
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Met Glu Val Gly Trp Tyr Arg Ser Pro Phe Ser Arg Val Val His Leu  
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Tyr Arg Asn Gly Lys  
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20